**α-Smooth muscle actin expression predicts the outcome of Kasai portoenterostomy in biliary atresia**

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**INTRODUCTION**

In biliary atresia (BA), a fibro-inflammatory process leads to the destruction of bile drainage pathways causing characteristic responses in the infant liver such as ductular proliferation, lobular inflammation, giant cell transformation, ductal plate malformation (DPM) and varying degrees of fibrosis. These responses provide us with information on the severity of liver disease at the time of presentation which may have an impact on the outcome of a Kasai portoenterostomy (KPE), which is the first line of treatment for BA. To identify factors that affect the outcome of KPE, we correlated relevant features in liver histology with outcome and we present our findings.

**Background/Aims:** Biliary atresia (BA) is a cholangio-destructive disease of the infant liver presenting with features of obstructive cholangiopathy. The Kasai portoenterostomy (KPE) is the first line of management. The aim of our study was to identify the characteristic features of liver histology in BA that impact the outcome of KPE.

**Patients and Methods:** Data from 30 consecutive children was retrieved from our prospectively maintained database of children undergoing KPE. This included basic demographics, laboratory values and histopathological data from liver biopsy. The stages of fibrosis, presence of ductal plate malformation (DPM), giant cell transformation, extramedullary hematopoiesis and area percentage of α-SMA (α-smooth muscle actin) expression was correlated with jaundice clearance after KPE using standard statistical tests. Native liver survival was computed.

**Results:** Overall, 13 (43%) children cleared jaundice in this series and 10 (33%) are alive with native liver. Lower area percent expression of α-SMA correlated with increased probability of jaundice clearance after KPE ($p < 0.001$). There was no correlation between stage of fibrosis and jaundice clearance ($p = 0.52$). DPM, giant cell transformation and extramedullary hematopoiesis did not correlate with outcome. All children who are alive with native liver had lower expression of α-SMA.

**Conclusion:** α-SMA expression may be a potential predictor of jaundice clearance and native liver survival after KPE.

**Keywords:** Biliary atresia, Kasai portoenterostomy, α-smooth muscle actin

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PATIENTS AND METHODS

Since 2013, we have maintained a prospective database of all children undergoing KPE in our institutions. A single surgeon performs all KPEs and a single pathologist reports the liver biopsy.

Collection of data

Basic demographics and preoperative laboratory values were collected for all children. Histopathological data from the liver biopsies of 30 consecutive children over a 3-year period was used in this analysis. These included stages of fibrosis, presence or absence of DPM like arrays, giant cell transformation, extramedullary hematopoiesis and area percentage of α-SMA positivity. These features were correlated with the success of the operation, defined as jaundice clearance with a decrease in serum direct bilirubin to <2 mg/dl, 6 months after KPE. Native liver survival was computed.

Histological examination

The Department of Pathology received wedge liver tissue obtained at the end of the procedure. The wedge liver was fixed in buffered formalin for 12–24 h. Thin sections measuring 4–5 µ were made from the paraffin-embedded material and stained with hematoxylin and eosin (H and E), Periodic acid-Schiff’s (PAS), PAS after diastase, Masson trichrome, orcein, iron and rhodanine.

Histologic grading of fibrosis

Paraffin-embedded liver sections were stained with hematoxylin and eosin stain to study fibrosis. A pathologist blinded to patient outcome scored these slides for degree of fibrosis. We used a 3-grade staging system previously used in BA[1] defined as mild (Stage I), if it ranged from portal fibrous expansion to bridging fibrosis involving <50% of portal tracts; moderate (Stage II), if bridging fibrosis >50% and severe (Stage III), if bridging fibrosis involved >50% of portal tracts and was accompanied by nodular architecture.

Other features of inflammation and fibrosis

The presence of circumferential biliary ductular structures within the connective tissue of portal tract in BA, which resemble primitive ductal structures were referred to as DPM like arrays. Other morphological features such as giant cell transformation and extramedullary hematopoiesis were also recorded.

Immunohistochemistry

Paraffin-embedded liver sections were processed together and incubated with a mouse monoclonal primary antibody directed against α-SMA (1:100, clone 1A4; Biocare, US). Bound antibodies were detected with biotinylated rabbit anti-mouse immunoglobulin G and a streptavidin–biotin complex/horseradish peroxide kit (DAKO Glostrup, Denmark) using 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical Co) as the chromogenic substrate. Sections were counter-stained with eosin.

Image analysis

A Leica DM microscope was used to acquire images of the sections. Portal tracts were randomly chosen in the liver, five from each slide in ×20/×40 magnification. The proportion of antibody positive area (area percent) was calculated for α-SMA with LAS Image Analysis software (Leica Microsystem). Slides were graded into three groups according to the area percent values. Grade I (mild) 0–4, Grade II (moderate) 5–9 and Grade III (severe) >10 [Figures 1-3]. The pathologist was blinded to the clinical outcome.

Statistical analysis

Continuous variables in normal distribution were expressed as means with standard deviation and analyzed using analysis of variance (ANOVA) test, those not in normal distribution were expressed as median with range and analyzed using the Mann–Whitney U test or Kruskal–Wallis test. Discrete variables were expressed as percentages and analyzed using a Chi-square test. A P < 0.05 was considered statistically significant. All data was collected after the approval from our Institutional Review Board.

RESULTS

There were 30 children in our study [Table 1]. Half (n = 15) were male. The median age at presentation was 83 days (range, 40–139 days). Mean preoperative total bilirubin was 10.1 ± 2.7 mg/dl. Median gamma-glutaryl transferase (GGT) was 718 IU/L (range, 111–2281 IU/L). Median aspartate alanine aminotransferase to platelet ratio index (APRI) calculated using the formula AST/upper limit of normal (ULN)/platelet count expressed as platelets ×10⁸/L ×100, was 1.24 (range, 0.36–8.72). 13 (43.3%) children had Stage I fibrosis, 9 (30%) had Stage...
II fibrosis and 8 (26.7%) had Stage III fibrosis. DPM was found in 15 (50%) patients. Median α-SMA percentage was 4.1% (range, 0.4–14.9%). α-SMA grading was mild in 20 (66.7%) children, moderate in 7 (23.3%) children and severe in 3 (10%) children. Giant cell transformation was seen in 16 (53.3%) children and extramedullary hematopoiesis was seen in 8 (26.7%) children.

Following KPE, 13 (43%) children cleared jaundice. There was no correlation between stage of fibrosis and jaundice clearance ($P = 0.52$). DPM (53.8% vs 47.1%, $P = 1.00$), giant cell transformation (61.5% vs 47.1%, $P = 0.48$) and extramedullary hematopoiesis (38.5% vs 17.6%, $P = 0.24$) also did not correlate independently with jaundice clearance after KPE.

A total of 12/20 children with mild α-SMA expression cleared jaundice compared to 1/10 who had moderate-to-severe α-SMA expression. Thus α-SMA area percentage was significantly lower in those who cleared jaundice (1.9%, range 0.4–8.4%) compared to those who failed to clear jaundice (5.5%, range 1.5–14.9%, $P = 0.001$). Higher stages of fibrosis had a higher percentage of α-SMA but this was not statistically significant (Stage I –3.5 ± 3.0%, Stage II –5.2 ± 4.2% and Stage III –6.1 ± 3.6%, $P = 0.27$). Age at KPE did not correlate with α-SMA grade (mild –95.5 ± 26.0, moderate –72.3 ± 32.5, severe –75.0 ± 15.7, $P = 0.12$). APRI did not correlate with α-SMA grade (mild –1.28, moderate –1.26, severe –0.64, $P = 0.37$).

Ten children who cleared jaundice and had mild expression of α-SMA are alive with native liver 6–27 months after KPE. One child who cleared jaundice but had moderate expression of α-SMA died of portal hypertension 6 months after KPE. Hence native liver survival was present only in children who cleared jaundice clearance and had mild α-SMA expression. All children who did not clear jaundice and had moderate-to-severe expression of α-SMA died or underwent liver transplantation.

![Figure 2: Grade II – α-smooth muscle actin area percent 7.093](image1)

![Figure 3: Grade III – α-smooth muscle actin area percent 12.615](image2)

**Table 1: Demographics and comparison of outcome**

| Variable                        | Total (n=30) | Successful KPE (n=13) | Failed KPE (n=17) | $P$  |
|---------------------------------|--------------|-----------------------|-------------------|------|
| Age at KPE, days                | 83 (40-139)  | 78 (40-139)           | 91 (42-134)       | 0.17 |
| Sex, male/female                | 15 /15       | 8 /5                  | 7/10              | 0.46 |
| Laboratory values               |              |                       |                   |      |
| Total bilirubin                 | 10.1±2.7     | 9.6±2.9               | 10.4±2.6          | 0.41 |
| GGT, IU/L                       | 718 (111-2281)| 695 (143-2281)       | 722 (111-1337)   | 0.97 |
| AST                             | 186 (65-543) | 206 (112-400)         | 186 (65-543)      | 0.80 |
| Platelet count, 1000 cells/m$^3$| 410.5±150.5  | 432.3±188.6           | 393.8±166.9       | 0.50 |
| APRI                            | 1.24 (0.36-8.72)| 1.21 (0.36-8.72) | 1.26 (0.37-3.09) | 0.71 |
| Histological features           |              |                       |                   |      |
| Fibrosis staging                |              |                       |                   |      |
| Stage I                         | 13 (43.3)    | 7 (53.8)              | 6 (35.3)          | 0.52 |
| Stage II                        | 9 (30.0)     | 4 (30.8)              | 5 (29.4)          | 0.52 |
| Stage III                       | 8 (26.7)     | 2 (15.4)              | 6 (35.3)          | 1.00 |
| DPM                             | 15 (50)      | 7 (53.8)              | 8 (47.1)          | 0.001|
| α-SMA percentage                | 4.1 (0.4-14.9)| 1.9 (0.4-8.4)        | 5.5 (1.5-14.9)    | 0.001|
| α-SMA                           |              |                       |                   |      |
| Mild                            | 20 (66.7)    | 12 (92.3)             | 8 (47.1)          | 0.03 |
| Moderate                        | 7 (23.3)     | 1 (7.7)               | 6 (35.3)          |      |
| Severe                          | 3 (10.0)     | 0 (0)                 | 3 (17.6)          |      |
| Giant cell transformation       | 16 (53.3)    | 8 (61.5)              | 8 (47.1)          | 0.48 |
| Extramedullary hematopoiesis    | 8 (26.7)     | 5 (38.5)              | 3 (17.6)          | 0.24 |

GGT: Gamma ‑glutaryl transferase, KPE: Kasai portoenterostomy, AST: Aspartate aminotransferase, APRI: AST to platelet ratio index, DPM: Ductal plate malformation, α-SMA: Alpha-smooth muscle actin
DISCUSSION

It is important to identify factors that affect the outcome of KPE. This helps us predict the prognosis of the operation and increases the possibilities of identifying targeted therapy to prevent disease progression. Liver histology has been the logical choice for several studies attempting to identify such factors. The classic histological findings of BA are fibrosis, DPM, giant cell transformation, extramedullary hematopoiesis, ductular proliferation, canalicular bile stasis, inflammation of the portal plate and lobular inflammation.

Fibrosis progresses with time from local to bridging and finally diffuse fibrosis and micronodular cirrhosis. However, in some studies there was no correlation between the extent of fibrosis and native liver survival. In our study 30% of children had Grade 2 fibrosis and 27% had Grade 3 fibrosis and this did not correlate with jaundice clearance.

DPM occurs due to failure of differentiation of the fetal biliary tract resulting in persistence of an excess of embryonic bile duct structures in the portal tracts. We have shown in our study of liver explants that the presence of DPM like arrays is associated with a shorter native liver survival. The incidence of DPM in our present series was 50%. Although some studies have reported that the presence of DPM in liver biopsies resulted in poor bile flow after KPE, in our study we found no correlation with jaundice clearance.

Giant cell transformation occurs due to the dissolution of cell membranes of adjacent hepatocytes and is seen in some other liver diseases in children like giant cell hepatitis, cholestatic liver disease and bile salt exporter protein defects. Giant cell transformation was seen in 53% of our patients. It has been shown that the presence of giant cell transformation was independently associated with the failure of KPE. But in our series, it did not correlate with jaundice clearance.

Extramedullary hematopoiesis did not have an independent correlation with native liver survival in a previous study. This was seen in 27% of children in our series but again it did not impact jaundice clearance after KPE.

Bile duct proliferation is a characteristic histological feature of BA. Ductular proliferation is the outcome of chronic cholestasis and results from the proliferation of preexisting intralobular bile ducts or because of transformation of periportal hepatocytes into ductules. Kinugasa et al. estimated ductular proliferation by CK 7 staining and reported that it correlated poorly with the outcome of KPE. Ductular proliferation, bile plugs, portal plate and lobular inflammation were seen in all liver biopsies in our series and hence we did not use these features to quantify the severity of liver disease.

Hepatic stellate cells (HSC) play an important role in liver fibrosis in BA. The activation of HSC and their transformation from fat-storing cells to myofibroblasts is responsible for increased type I collagen production in BA. HSC activity is determined by the expression of an intracellular microfilament protein called α-smooth muscle actin (α-SMA). Shteyer et al. studied α-SMA expression in the portal tracts as well as lobules of the liver and reported that degree of α-SMA expression and fibrosis scores predict the outcome of KPE such as jaundice clearance and native liver survival. In their series, fibrosis scores were also significantly lower with lower α-SMA expression. Dong et al. also negatively correlated α-SMA expression with the reduction in direct bilirubin 3 months after KPE. In our series, we only measured α-SMA activity in portal areas and excluded the expression in sinusoidalstellate cells. α-SMA scores expressed as area percent significantly correlated with jaundice clearance after KPE. Infants with a lower area percent of α-SMA had better jaundice clearance. In our series, 20 children had mild expression of α-SMA compared to 8 and 2 children, respectively, in the above mentioned studies. It is important to note from our study that survival with native liver was seen only in children with mild α-SMA expression. In long-term survivors with BA, disappearance of peri-ductal α-SMA has been reported after a successful KPE and its expression was higher in patients who underwent LT by the age of 2 years. Unlike previous reports, liver fibrosis and APRI which is a predictor of the extent of liver fibrosis did not correlate significantly with α-SMA expression in our study. We used the same staging system as these studies, but the clone of antibody was different.

Liver histology has been shown to have a limited value in predicting the outcome of KPE. This was the case in our series also. However, α-SMA expression may be a potential marker for predicting the outcome of the operation. Recently microRNA-21 was found to be upregulated in BA. This activates the pathways which provoke liver fibrosis by enhancing α-SMA. MicroRNA-19b has been shown to down-regulate HSC activation which was confirmed by decreased α-SMA expression and type I collagen expression. These can be used in the future for targeted therapy to improve the prognosis of BA.

We intend to perform α-SMA area percent estimation in all wedge liver tissue obtained from infants at the time of explant.
KPE and study its prognostic accuracy in larger numbers. We also intend to follow up our patients to compute native liver survival and correlate it with α-SMA expression.

CONCLUSION

Among all the histological factors analyzed, α-SMA alone has the probable potential to predict the jaundice clearance and native liver survival after KPE.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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