

Insulin-like Growth Factor-1 (IGF-1) in Children with Postoperative Biliary Atresia: A Cross-Sectional Study

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SUMMARY Biliary atresia is the leading cause of chronic infantile cholestasis which eventually leads to cirrhosis. Re-establishment of biliary drainage by Kasai portoenterostomy and liver transplantation for end-stage liver disease has favorably altered the clinical outcome. However, growth failure, one of the major complications of chronic liver disease, remains a major problem. The aim of the study is to evaluate growth, nutritional status and serum growth factor IGF-1 in children with biliary atresia after Kasai operation and at comparing these data between the groups with successful and unsuccessful operation. Fifty-four children with postoperative biliary atresia were evaluated for their clinical outcome, height, blood biochemistry related nutritional status and serum IGF-1. Height and serum IGF-1 were expressed as standard deviation score (SDS) to minimize the influence of age. With 44.4% of the enrolled patients the operation had been unsuccessful and jaundice persisted. The mean age of children with jaundice in comparison with the jaundice free groups was not significantly different (42.0 and 49.9 months, $p = 0.458$). In jaundice-free patients, hematocrit, serum albumin, calcium and phosphorus were normal and significantly higher. In the successful Kasai group, the height SDS and serum IGF-1 SDS were within the normal range and significantly higher (height SDS 0.2 ± 1.0 vs. -0.9 ± 1.2 , $p < 0.01$ and IGF-1 SDS 0.5 ± 2.2 vs. -1.3 ± 1.0 , $p < 0.01$). The mean IGF-1 SDS in the failed Kasai group was less than -1 . Children with good outcome of postoperative biliary atresia showed better growth, better nutritional status and higher serum IGF-1 levels when compared to those with unsuccessful operation.

Biliary atresia is the leading cause of chronic infantile cholestasis. The disease results from a progressive inflammatory and fibro-obliterative process of the extrahepatic biliary tree that finally results in impaired bile flow, chronic cholestasis, hepatocellular injury and biliary cirrhosis. The incidence of biliary atresia is approximately one in 10,000 live births worldwide.¹ There appears to be a higher incidence in Asian and Pacific countries than in Western countries.^{2,3} Several mechanisms have been postulated to explain the pathology of this disease but the exact mechanism is still unknown. Some studies have reported seasonal clustering supporting the theory of environmental factors such as viral exposure during

the perinatal period.⁴ Surgical management by Kasai portoenterostomy to re-establish biliary drainage and liver transplant for end-stage liver disease has produced favorable outcomes. The approximate 5- and 10-year survival rates of patients who had undergone Kasai portoenterostomy were 35 to 60% and 25 to 35% with their own liver, respectively,^{1,5,6} whereas the remainder would require liver transplantation. Patients with inadequate bile flow following porto-

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terostomy will eventually develop progressive fibrosis, cirrhosis and growth failure.

Normally, a child's growth depends on good nutrition, growth hormone and growth factors such as insulin-like growth factors (IGFs). Most anabolic actions of growth hormone are mediated through insulin-like growth factor-1 (IGF-1). IGF-1 plays an important role in the regulation, differentiation and proliferation of many cell types resulting in human growth. Over 50% of children with established liver cirrhosis show evidence of growth failure and malnutrition. The liver is crucial in that it produces IGF-1 and its major binding proteins (IGFBP-1, -2 and -3). In children with chronic liver disease, the circulating IGF-1, -2 and IGFBP-3 levels are low despite an increment in growth hormone secretion.⁷⁻⁹ After orthotopic liver transplantation, improvements in the GH/IGF-1 ratio and growth are observed.^{8,9} Yet, growth hormone therapy can not promote the anabolic effect and increase the IGF-1 levels in children with biliary atresia and chronic liver disease awaiting liver transplantation.^{10,11}

In this study, we evaluated growth, nutritional status and serum IGF-1 levels in children with biliary atresia who had undergone Kasai portoenterostomy. We also compared the growth and serum IGF-1 levels between the groups with successful and unsuccessful Kasai portoenterostomy.

MATERIALS AND METHODS

The protocol of the study was approved by the Ethics committee, Faculty of Medicine, Chulalongkorn University. The children's parents were informed as to the objectives of the study and their written consent was obtained. Blood samples were taken during a routine biochemical evaluation service. Sera were separated and stored at -70°C until further tested.

Fifty-four biliary atresia patients who had undergone Kasai portoenterostomy at King Chulalongkorn Memorial Hospital were enrolled in the study. All patients were evaluated at the Pediatric Gastroenterology Outpatient Clinic for weight, height, clinical outcome after surgery, blood biochemistry including nutritional status and serum IGF-1.

The blood samples were collected from all patients to determine blood biochemistry including complete blood count (CBC), bilirubin, albumin, globulin, calcium, magnesium, BUN, creatinine and prothrombin time. Serum IGF-1 was determined by ELISA, using the OCTEIA^R IGF-1 kit which is a two-site immunoenzymometric (IEMA) assay for the quantitative determination of IGF-1 in human serum or plasma. The patients' samples were briefly incubated with a releasing agent to activate binding proteins, and then diluted for the assay. In the OCTEIA^R kit, a purified sheep polyclonal anti-IGF-1 has been coated onto the inner surface of polystyrene microtitre wells (the solid phase or capture antibody). The pre-treated, diluted sample was then incubated, together with horseradish peroxidase-labelled monoclonal anti-IGF-1, in the antibody coated well at room temperature for 2 hours. The wells were washed and a single component chromogenic substrate was added to develop color. The absorbance of the stopped reaction mixture was read in a microtitre plate reader, with the color intensity developed being directly proportional to the amount of IGF-1 present in the sample.

The serum IGF-1 levels were compared to the normal value in Thai children.^{12,13} The results of the measured IGF-1 were converted into standard deviation score (SDS) according to the formula

$$\text{SDS} = \frac{X - \text{average } X}{\text{SD}}$$

where X is the observed value, average X is the mean of the normal value at the respective age and SD is the standard deviation from the mean.

Height and weight of all patients were determined and compared to the normal growth rates of Thai children.¹⁴ These results were also converted to standard deviation score (SDS) according to the above formula.

Successful Kasai portoenterostomy was defined as the good outcome of biliary drainage which resulted in disappearance of clinical jaundice and total bilirubin below 2 mg/dl.

Statistical Analysis

The results were presented as mean, standard deviations and standard deviation scores. T test was

used to compare between two groups with successful and unsuccessful portoenterostomy, respectively. A p -value < 0.05 was considered significant.

RESULTS

There were 54 patients enrolled in the study (25 males and 29 females) with their ages ranging from 1 to 16 years. All but three cases had undergone Kasai portoenterostomy. Most of them (46 out of 54) had been subjected to the operation within the first three months of age. On three cases only an intraoperative cholangiogram was performed to confirm the diagnosis as they were beyond five months of age. These patients were classified as being part of the unsuccessful operation group.

There were 30 successful operations (55.6%) and 24 unsuccessful operations (44.4%). The mean age was 42.0 ± 25.0 and 49.9 ± 50.6 months in the successful and unsuccessful operation groups, respectively ($p = 0.458$). As for growth parameters, the height was converted into standard deviation score (SDS) according to the age group. The mean height SDS was significantly lower in the jaundice group than in the successful operation group (-0.9 ± 1.2 vs. 0.2 ± 1.0 , $p = 0.0003$). The actual weight could not be evaluated accurately due to some interference factors, especially ascites, in some patients.

The blood biochemistry is shown in Table 1. Hematocrit, calcium, albumin, globulin and phosphorus were significantly different between the groups.

The serum IGF-1 was converted into the IGF-1 SDS by using the levels obtained from normal Thai children as shown in Table 2. The IGF-1 SDS of the jaundice group was lower than -1 SDS and was significantly lower than that of the successful Kasai group ($p < 0.01$).

DISCUSSION

Most of the patients had undergone Kasai portoenterostomy at the appropriate time. However, nearly half of the enrolled patients had an unfavorable outcome with persistent jaundice which eventually would turn into liver cirrhosis. One of the significant complications of childhood chronic liver disease is growth failure. Anthropometric parameters and some biochemical data were used to evaluate growth and nutrition status. Height standard deviation score was used to minimize the influence of age and to facilitate comparison between the two groups.

The nutritional status in jaundice-free biliary atresia patients was good. Height, a measure that might reflect the long term nutrition status, did not

Table 1 Demographic, biochemistry data and IGF1 SDS of the 2 study groups

	Successful portoenterostomy		Unsuccessful portoenterostomy		<i>p</i>
	<i>n</i>	Mean \pm SD	<i>n</i>	Mean \pm SD	
Age (months)	30	42.0 \pm 25.0	24	49.9 \pm 50.6	0.458
Height SDS	27	0.2 \pm 1.0	23	-0.9 \pm 1.2	0.0003
Hematocrit (%)	30	35.0 \pm 3.1	22	30.1 \pm 4.1	< 0.01
Platelet ($\times 10^3$ /cu.mm)	30	210.9 \pm 102.9	22	160.8 \pm 93.3	0.077
Calcium (mg/dl)	30	9.7 \pm 0.9	23	9.2 \pm 0.8	0.03
Albumin (g/dl)	30	4.6 \pm 0.4	23	3.5 \pm 0.6	< 0.01
Globulin (g/dl)	30	3.1 \pm 0.5	23	3.9 \pm 1.1	< 0.01
Prothrombin time (seconds)	28	13.1 \pm 2.1	23	14.5 \pm 3.8	0.09
Cholesterol (mg/dl)	30	192.4 \pm 63.6	21	229.2 \pm 117	0.16
Magnesium (mmol/l)	29	0.9 \pm 0.2	23	1.1 \pm 1.9	0.141
Phosphorus (mg/dl)	30	5.5 \pm 0.8	23	4.5 \pm 0.8	< 0.01
IGF-1SDS	29	0.45 \pm 2.2	24	-1.313 \pm 1.0	< 0.01

SDS, standard deviation score

deviate from the normal population. In contrast, there was significant growth retardation in the jaundice group. The biochemical parameters representing nutritional status and liver synthetic function were also better in the jaundice-free group. It appeared likely for a patient with a successful operation to grow like a normal child.

It has been established that growth depends on good nutrition, growth hormone and growth factors. The liver is the essential organ for production of growth factors which play a major role in body growth. Enhancement of growth by improving nutrition via nasogastric feeding could not normalize the circulating IGF-1 and IGF-BPs.¹⁵ Children with biliary atresia awaiting liver transplantation are insensitive to growth hormone and the treatment is unlikely to promote the anabolic effect.¹⁰ Low bone mineral density was also observed in association with low levels of serum 25-hydroxyvitamin D and serum IGF-1 in children with biliary atresia after unsuccessful Kasai operation.¹⁶ Liver transplantation could reverse the low bone mass and lead to an increase in serum 25-hydroxyvitamin D and IGF-1.¹⁶ There have been many reports on improvement in growth, growth hormone and IGF-1 after orthotopic liver transplantation in children with end-stage liver disease.^{8,9,16}

Our results have shown that jaundice-free patients had normal levels of serum IGF-1 compared to the reference parameters of the same age group whereas jaundice patients had lower serum IGF-1 and less than -1 SDS. The IGF-1 SDS of -1 and IGF-BP-3 SDS of -1.3 have been previously reported to be used as a cut-off point to help diagnose growth hormone deficiency in patients with short stature.¹³ In biliary atresia, the liver which produces IGF-1 is

impaired and growth hormone therapy cannot improve growth. Patients with unsuccessful Kasai operation would display growth retardation, malnutrition and significantly lower levels of serum IGF-1 and other biochemical markers related to their nutritional status.

In conclusion, children with biliary atresia after successful Kasai portoenterostomy developed normal growth, nutritional status and serum IGF-1 levels. In contrast, unsuccessful operation might result in chronic liver disease leading to growth failure, malnutrition, abnormal blood biochemistry and lower serum IGF-1 levels.

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Table 2 Serum IGF-1 levels among 5 different age groups of healthy Thai children¹²

Age (years)	n	Mean (ng/ml)	SD (ng/ml)
0-4	12	51.5	32.4
4-8	21	144.2	74.1
8-10	21	222.7	120.4
10-12	15	349.1	226.4
12-15	18	553.8	271.9

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