HK J Paediatr (new series) 2024;**29**:82-92

Original Article

The Effects of Early Enteral Nutrition on the Prognosis of Children with Biliary Atresia After the Kasai Procedure

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Abstract

Objectives: This study aimed to determine the efficacy of early enteral nutrition in the treatment of jaundice and improving the liver function, intestinal flora and nutritional status of children after portojejunal anastomosis (Kasai) towards reducing the incidence of cholangitis. ***Study Design:*** A case-control study. ***Place and Duration of Study:*** Shenzhen Children's Hospital from August 1st, 2020 to December 31st, 2021. ***Methods:*** A prospective randomised controlled study was performed in children diagnosed with biliary atresia who underwent the Kasai procedure. The patients were randomised to a control group that received total parenteral nutrition and an experimental group that received early enteral nutrition. The patients were followed up for 1 year and the liver function indices, intestinal flora, growth and nutritional status were analysed. The time to the first postoperative incidence of cholangitis, the number of cholangitis attacks and the survival rate of the autologous liver at one year were compared across the two groups. ***Results:*** At thirty and sixty days after the operation, the survival of patients in the experimental group was significantly higher than in the control group (P<0.05). At 7, 30 and 60 days after the operation, the levels of red blood cells, white blood cells and peripheral blood cells were significantly higher in the experimental group compared to the control group (P<0.05). At 30 and 60 days after the operation, the levels of total bilirubin and direct bilirubin were significantly lower in the experimental group compared to the control group (P<0.05). At 7, 30 and 60 days after the operation, the levels of aspartate aminotransferase, alanine aminotransferase and γ-glutamyltransferase, alkaline phosphatase and total bile acid were all lower in the experimental group compared to the control group (P<0.05). No significant differences in the numbers of *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Escherichia coli* in the feces were observed between the 2 groups before the operation (P>0.05). Before recovery with oral feeding, the numbers of *Bifidobacterium* and *Lactobacillus* in the feces of the experimental group were higher than the control group. Also, the numbers of *Enterococcus* and *Escherichia coli* in the experimental group were lower than in the control group (P<0.05). Before recovery with oral feeding, the levels of fecal bacteria in the experimental group were significantly higher than the control group

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Received February 2, 2022

(P<0.05). The average times to the first postoperative incidence of cholangitis in the experimental group were longer than that in the control group. The average number of cholangitis incidents was significantly lower in the experimental group compared to the control group (P<0.05). ***Conclusions:*** Early enteral nutrition can improve the nutritional status and growth of children following the Kasai procedure that is conducive to postoperative recovery. It can also improve the gastrointestinal flora balance, reduce the number of postoperative cholangitis attacks and improve the survival rate of autologous liver. Our data are of particular significance to improving the management of children with biliary atresia.

Key words *Autologous liver survival; Biliary atresia; Early enteral nutrition; Intestinal bacteria; Kasai procedure*

Introduction

Biliary atresia (BA)[1] is characterised by progressive inflammation of the intra- and extrahepatic bile ducts, fibrous obstruction and cholestasis that frequently occurs in infants and young children. These manifestations can cause progressive liver fibrosis and cirrhosis that eventually lead to liver failure and death.[2,3] In Asia, the incidence of BA is 1.5 in 10,000.[4] Kasai portoenterostomy is the preferred surgical treatment for children with BA. The success rate of the Kasai procedure determines the survival time of children with autologous liver disease and directly impacts the prognosis of children.[5,6] Long-term clinical evidence has shown that poor recovery after Kasai surgery directly affects the growth, quality of life, survival and mortality of children and results in major societal burden.[7] Also, the age at the time of the operation, poor regression of jaundice, lack of liver function recovery and complicated cholangitis are independent factors that influence the postoperative efficacy of Kasai surgery in children.[8,9]

Cholangitis[10] is one of the most common complications after the Kasai procedure that usually occurs within 1 year after the operation with an incidence of 30-60%.[7] Cholangitis also frequently recurs. A previous study[11] showed that recurrent cholangitis is an independent risk factor for death within 3 years after the Kasai operation. Recurrent cholangitis after the Kasai operation can be divided into early and late stages. Early-stage cholangitis mostly within 1 month after the operation and is characterised by obstruction of the bile duct and recurrence resulting in death. Late-stage cholangitis can cause portal hypertension and cirrhosis.[12] Children with BA are often malnourished with poor immune function and growth retardation.[13] Patients also have abnormal digestive tract function that can be further aggravated leading to postoperative complications.[14] Cholangitis is known to be related to the migration of intestinal microbes caused by long-term fasting and so nutritional therapy is particularly important in children with BA after the Kasai operation.[15] Clinically, nutritional therapy can be divided into parenteral and enteral nutrition. Generally, single or combined nutrition therapy is adopted based on the gastrointestinal function of children. Compared to parenteral nutrition, enteral nutrition is more suitable for human physiological conditions and is economical and safe. The purpose of this study was to determine if early enteral nutrition (EEN) could improve the nutritional status of children with BA after the Kasai operation or promote the recovery of liver function and improve the intestinal flora. This approach may lead to a reduction in the incidence of cholangitis.

Data and Methods

Subjects

The clinical data of 57 children with BA who underwent hepaticojejunostomy in the Department of General Surgery of the Shenzhen Children's Hospital from August 1st, 2020 to December 31st, 2021 were analysed in this study. Patients in the cohort were divided into a control group (n=22) and an experimental group (n=25). The inclusion criteria were as follows;[1] Children in line with the diagnostic criteria in the Practice Guideline for Diagnosis and Treatment of Biliary Atresia in Mainland China (Expert Consensus) and who underwent Kasai portoenterostomy after diagnosis of BA.[2] Children and their families who cooperated with the doctors for treatment and agreed to take drugs after the operation and periodic review to acquire follow-up data.

The exclusion criteria were as follows;[1] Children with severe diseases of the kidneys, blood, endocrine, cardiovascular and nervous system.[2] Children with liver cirrhosis complicated with hepatolithiasis, portal hypertension, and gastrointestinal bleeding.[3] Children who could not cooperate with doctors and did not agree to take drugs or undergo periodic review or had incomplete follow-up data.

No significant differences in age, gender, gestational age, body weight and other general information were observed between the two groups (all P>0.05; Table 1). This study was approved by the medical ethics committee of the hospital and patients were recruited under written informed consent. This research was supported by the paediatric nutrition support team and developed through the scientific research project (No. xm-2019-000-0114-03), the Guangdong Medical Research Fund (No. A2019541) and the Sanming Project of Medicine in Shenzhen (No. SZSM201812055).

Treatment Methods

All children underwent conventional treatment according to the *Diagnosis and Treatment of Biliary Atresia in Mainland China (Expert Consensus)* after the Kasai operation. The nutrition support team was composed of doctors, nutritionists, nurses and pharmacists. On the first day of admission, the children underwent body mass, nutritional and immune indicator detection, and STAMP nutritional risk screening. The nutritional regimen after the operation was: calorie of 108 kcal/kg, protein of 2.2 g/kg, and an energy nitrogen ratio of 100-200 kcal:1g (1 kcal = 4.184 kJ). Nutritional support was given according to the standard recommendations and the course of treatment was 7-10 days. The total energy intake of the two groups was the same during 7-10 days after the operation.

Patients in the experimental group were treated with EEN support. During the operation, the operator placed the nasal jejunal feeding tubes 15-20 cm away from the gastrointestinal anastomosis. On the first day after the operation, the nasointestinal tube was located away from the anastomosis. After 48 hours, isosmotic sugar saline was infused at a rate of 2-3 mL/(kg·h). After 24 hours with no reported discomfort, a semi-element infant formula rich in medium-chain triglyceride (ALFARE, Nestle Corporation, containing 282 kJ of calories, 1.9 g of protein, 7.3 g of sugar and 3.4 g of fat per 100 mL) was infused at a rate of 1-2 mL/(kg·h). After adaptation, the dosage was gradually increased by 10-20 mL/(kg·d) and the target infusion rate was 20-50 mL/h. During the period of enteral nutrition, due to the special dynamic effects of food, it was found that the calorie intake was 10% more than the standard recommendations. In the first few days when the amount of heat and nitrogen was insufficient and did not reach 70% of the physiological requirement, short-term intravenous nutrition was supplemented and enteral nutrition was stopped after the children returned to total oral feeding.

Patients in the control group were treated with total parenteral nutrition (TPN) support. From 24 hours after the operation, the children were given TPN. The heat supply was calculated as non-protein heat. Long-chain fat emulsion provided 40% of the calories and glucose provided 60% of the calories. The amount of nitrogen supplement was 1.5-2.5 g/(kg·d) and the main nitrogen source was 19AA paediatric amino acids (60% essential amino acids, 30% branched-chain amino acids and a certain amount of arginine). After the recovery of gastrointestinal function, oral feeding was implemented to gradually reach the physiological requirements. TPN was stopped after the children had returned to total oral feeding.

Observational Indices

(1) Growth and development of children: The growth and development of children in the two groups on the 1st day of admission and on the 7th, 30th and 60th days after the operation were compared. These measures included the weight, length, upper arm circumference, the tricep skin fold, upper arm area, upper arm muscle fat area and the fat index.

(2) Nutritional status: Fasting venous blood was collected on the 1st day of admission and on the 7th, 30th and 60th days after the operation. The laboratory tests included serum albumin (Alb), serum globulin (GLO), total cholesterol (TC), red blood cell count (RBC), white

Table 1 Summary of the general characteristics of the patients

	Gender (Male/Female)	Gestation (Week)	Age of operation (Day)	Weight (kg)
Control group (n=22)	10/12	38.41±1.51	59.54±10.34	5.14±0.67
Experimental group (n=25)	11/14	38.27±1.62	60.29±11.16	5.10±0.88
χ^2/t	0.0100	0.3364	0.2624	0.1949
P	0.9203	0.7378	0.7939	0.8461

blood cell count (WBC), blood platelet count (BPC) and blood glucose (BG). Serum albumin is one of the most commonly used serum markers of nutritional status.

(3) Liver function recovery and jaundice subsidence: Direct bilirubin (DBil) and total bilirubin (TBil) were detected to determine the jaundice subsidence time. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltransferase (γ-GT), alkaline phosphatase (ALP), plasma prothrombin time (PT), activated partial thromboplastin time (APTT), urea nitrogen (BUN) and creatinine (CR) were detected to determine the recovery kinetics of liver function in the children.

(4) Intestinal microecology: The feces of the two groups were collected before the operation, on the 1st day after the operation and before oral feeding to determine the proportion of bacteria in the feces. The intestinal excreta of the two groups were cultured.

The bacterial count of *Bifidobacterium, Lactobacillus, Enterococcus* and *Escherichia coli* was calculated by a viable plate count method. Briefly, 0.5 g of fresh feces from the children in each period were sampled and placed into test tubes containing 4.5 mL of phosphate-buffered saline, The samples were mixed and serially diluted from a concentration of 10^{-1} to 10^{-7}. 10 μL of each dilution was inoculated onto a selective medium plate. After inoculation, aerobic bacteria were cultured at 37°C for 24 hours and anaerobic bacteria were cultured at 37°C in an anaerobic incubator for 72 hours.

Fluorescent quantitative Polymerase Chain Reaction (PCR) was used to detect the proportion of bacteria in the samples. Fresh feces samples were pretreated and fecal bacterial DNA was extracted according to the instructions of the fecal bacterial genomic DNA Extraction Kit (ThermoFisher). The primer sequence is shown in Table 2. The PCR reaction conditions were denaturation at 95°C for 5 minutes, 95°C for 15 seconds, 50°C for 1 minute, 72°C for 45 seconds and 87°C for 5 seconds, for 40 cycles.

(5) Postoperative follow-up: The two groups were followed up for 1 year. The time to the first incident of postoperative cholangitis, the number of cholangitis incidents and the survival rate of autologous liver within 1 year were recorded. The diagnostic criteria for cholangitis included the exclusion of other diseases or a high fever that could not be explained by other reasons; aggravation or recurrence of scleral yellow staining of the skin; lightening of stool colour or recurrence of clay colour stool; serum DBil and TBil increase; blood routine examination showing significantly increased leukocytes and neutrophils. Outcome events were defined when a child had died or had to undergo liver transplantation. The survival time was defined as the time from the start of the study to relapse or death for any reason.

Statistical Analysis

Statistical analysis was performed using SPSS22.0 software. The measurement data were tested for normality and homogeneity of variance. A one-way ANOVA of the mean ± SD and an independent sample t-test was used to analyse normally distributed data with homogeneous variance. A rank-sum test was used to analyse data that was not normally distributed. A Chi-square test was used for counting data. *P*-values <0.05 were set as statistically significant.

Table 2 Summary of the primer sequences

	PCR Fragment Length（bp）	Primer Sequence
Enterococcus Thiercelin and Jouhaud	154	F：5'-CCCTTATTGTTAGTTGCCATCATT-3'
		R：5'-ACTCGTTGTACTTCCCATTGT-3'
Escherichia	344	F：5'-CTCGCTGGCATTTGCGTAC-3'
		R：5'-ATCTTTTGCCGTCCGTTTTGG-3'
Bacteroides	169	F：5'-TGACAGTGAGAGATTGCTGCGTT-3'
		R：5'-TCAGCCCACATTTCCTCTTCCGT-3'
Bifidobacterium	244	F：5'-TCGCGTCGGTGAAAG-3'
		R：5'-CCACATCCAGCGTCCAC-3'
Lactobacillus	300	F：5'-AGCAGTAGGGAATCTTCCA-3'
		R：5'-CACCGGTCACATGCAG-3'
Clostridium Prazmowski	260	F：5'-CGCAGAAGGTGAAAGTCCTGTAT-3'
		R：5'-TGGTCCTCACTGATTCACACAGA-3'
Fusobacterium	100	F：5'-CGCAGAAGGTGAAAGTCCTGTAT-3'
		R：5'-TGGTCCTCACTGATTCACACAGA-3'

Results

Growth and Development of the Two Groups

The growth and development of patients in the two groups were observed on the 1st day after admission and on the 7th, 30th and 60th days after the operation. The results showed no significant differences in weight, length, upper arm circumference, tricep skinfold and BMI between the two groups (all P>0.05) on the 1st day of admission. On the 7th, 30th and 60th days after the operation, the growth indices of the two groups improved but on the 7th day after the operation, the BMI of patients in the experimental group was significantly higher than the control group (P<0.05). No significant differences were observed in the other indices between the two groups (P>0.05). On the 30th and 60th days after the operation, the weight, upper arm circumference, triceps skin wrinkles and the BMI patients in the experimental group were significantly higher than in the control group (P<0.05). No significant differences in body lengths were observed between the two groups (P>0.05; Table 3).

Nutritional Status

The nutritional status of the two groups was observed on the 1st day after admission and on the 7th, 30th and 60th days after the operation. No significant differences in ALB, GLO, TC, RBC, WBC and peripheral blood cells (PBC) were observed between the two groups on the first day of admission (all P>0.05). The levels of ALB, GLO, RBC, WBC and PBC increased and TC decreased in the two groups on the 7th, 30th and 60th days after the operation. No significant differences in the levels of ALB, GLO and TC were observed between the two groups at each stage (P>0.05). On the 7th, 30th and 60th days after the operation, the levels of RBC, WBC and PBC in the experimental group were significantly higher than in the control group (all P<0.05; Table 4).

TBil and DBil Levels in Two Groups

On the 1st day of admission, no significant differences in the levels of TBil and DBil were observed between the two groups (both P>0.05) suggesting that the children had jaundice. On the 7th, 30th and 60th days after the operation, the levels of TBil and DBil in the two groups gradually decreased. On the 30th and 60th days, the levels of TBil and DBil in the research group were significantly lower than in the control group (both P<0.05) suggesting that the jaundice of all the patients had gradually subsided (Figure 1).

Table 3 Summary of the growth and development of the patients in the two groups

	1st day after admission				
	Weight (kg)	**Length (cm)**	**Upper arm circumference (cm)**	**Tricep skinfold (mm)**	**BMI (kg/m²)**
Control group (n=22)	5.14±0.67	52.48±2.84	8.56±0.71	6.12±0.25	18.51±0.59
Experimental group (n=25)	5.10±0.88	53.01±2.97	8.62±0.67	6.23±0.31	18.44±0.70
χ^2/t	0.1735	0.6230	0.2979	1.3269	0.3678
P	0.8631	0.5364	0.7671	0.1912	0.7147
	7 days after the operation				
Control group (n=22)	5.38±0.59	53.25±2.35	8.83±0.59	6.26±0.29	18.77±0.37
Experimental group (n=25)	5.51±0.44	53.67±2.08	9.01±0.62	6.34±0.32	19.03±0.35
χ^2/t	0.8627	0.6500	1.0158	0.8933	2.4742
P	0.3928	0.5189	0.3151	0.3764	**0.0172**
	30 days after the operation				
Control group (n=22)	6.02±0.61	56.87±2.08	9.13±0.81	6.84±0.25	18.97±0.41
Experimental group (n=25)	6.35±0.51	57.33±1.89	9.84±0.87	7.03±0.28	19.23±0.36
χ^2/t	2.0198	0.7944	2.8827	2.4396	2.3153
P	**0.0494**	0.4311	**0.0060**	**0.0187**	**0.0252**
	60 days after the operation				
Control group (n=22)	7.27±0.91	60.14±2.47	10.06±1.12	7.16±0.44	20.08±0.56
Experimental group (n=25)	7.81±0.83	61.33±2.19	10.77±1.01	7.51±0.32	20.75±0.49
χ^2/t	2.1275	1.7509	2.2854	3.1446	4.3754
P	**0.0389**	0.0867	**0.0270**	**0.0029**	**<0.0001**

Recovery of Liver Function in Two Groups

On the 7th, 30th and 60th days after the operation, the levels of AST, ALT, γ-GT, ALP and TBA in the experimental group were lower than on the 1st day of admission and lower than those in the control group ($P<0.05$, Table 5). These data suggest that liver function had improved more in the research group compared to the control group.

Bacterial Counts Detected by Plate Culture

There was no significant difference in the number of *Bifidobacterium, Lactobacillus, Enterococcus and*

Table 4 Summary of the nutritional status of the two groups

	Alb (g/L)	GLO (g/L)	TC (mmol/L)	RBC (×10¹²/L)	WBC (×10⁹/L)	PBC (×10⁹/L)
1st day after admission						
Control group (n=22)	35.84±5.79	16.34±3.37	5.51±0.36	3.12±0.31	4.05±0.68	134.61±9.68
Experimental group (n=25)	36.21±6.34	15.97±3.69	5.48±0.39	3.16±0.35	4.13±0.75	139.43±10.08
χ^2/t	0.2078	0.3571	0.2727	0.4122	0.3810	1.6663
P	0.8363	0.7227	0.7863	0.6821	0.7049	0.1026
7 days after the operation						
Control group (n=22)	36.24±4.63	23.15±1.68	5.25±0.32	3.76±0.19	4.69±0.57	151.67±10.26
Experimental group (n=25)	37.59±5.02	23.70±1.93	5.21±0.29	3.91±0.23	4.97±0.32	162.17±9.77
χ^2/t	0.9538	1.0351	0.4495	2.4173	2.1092	3.5913
P	0.3453	0.3061	0.6552	**0.0197**	**0.0405**	**0.0008**
30 days after the operation						
Control group (n=22)	39.84±4.23	25.97±1.77	4.97±0.31	4.07±0.40	5.81±0.61	218.64±15.24
Experimental group (n=25)	40.28±4.87	26.16±2.03	4.90±0.39	4.38±0.21	6.18±0.46	234.35±12.84
χ^2/t	0.3285	0.3397	0.6747	3.3843	2.3647	3.8356
P	0.7441	0.7356	0.5033	**0.0015**	**0.0224**	**0.0003**
60 days after the operation						
Control group (n=22)	40.56±2.08	30.15±2.97	4.61±0.28	4.41±0.33	6.43±1.03	279.51±21.64
Experimental group (n=25)	41.18±2.67	31.37±3.38	4.53±0.33	4.94±0.20	7.11±0.87	303.76±20.31
χ^2/t	0.8791	1.3062	0.8895	6.7496	2.4537	3.9613
P	0.3840	0.1981	0.3784	**<0.0001**	**0.0181**	**0.0002**

Alb: albumin; GLO: globulin; TC: total cholesterol; RBC: red blood cell; WBC: white blood cell; PBC: peripheral blood cells

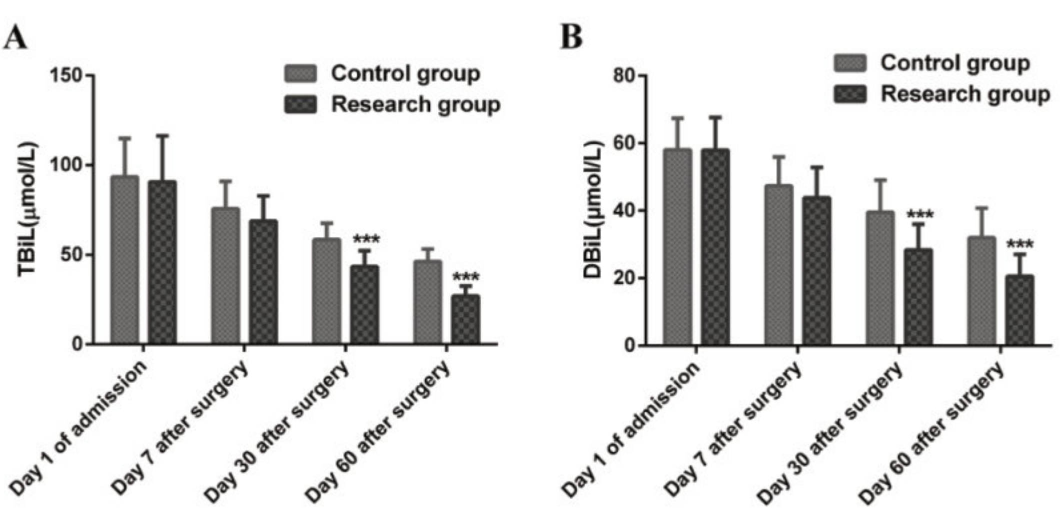

Figure 1 The TBil and DBil levels in the two patient groups.

Escherichia coli in feces between the two groups before operation (all P>0.05). On the 1st day after the operation, the number of *Bifidobacterium* and *Lactobacillus* in the feces of the two groups increased, while the number of *Enterococcus* and *Escherichia coli* decreased, with no significant difference between the two groups (all P>0.05). Before the recovery of oral feeding, the number of *Bifidobacterium* and *Lactobacillus* in the feces of the experimental group was higher than in the control group, and the number of *Enterococcus* and *Escherichia* coli in the feces of the experimental group was significantly lower than the control group (P<0.05, Table 6).

Fluorescence Quantitative Sample Analysis

The levels of Enterococcus *Thiercelin and Jouhaud* and *Escherichia* in the intestinal flora of the two groups decreased with time before the operation, on the 1st day after the operation and before recovery with oral feeding whilst the levels of *Bacteroides, Bifidobacterium, Lactobacillus, Clostridium Prazmowski* and *Fusobacterium* increased. Before and on the 1st day after the operation, there were no significant differences in the levels of bacteria between the two groups (all P>0.05). Before recovery with oral feeding, the levels of bacteria in the experimental group were significantly higher than in the control group (all P<0.05; Figure 2).

Follow Up

At one year follow-up, the number of children complicated with cholangitis was not significantly different between the control (10 children) and the experimental groups (8 children) (P>0.05). However, the first time of the initial cholangitis incident in the research group was significantly longer than the control group and the attack frequency was significantly lower than that in the control group (P<0.05). There was no significant difference in the survival rate of autologous liver at one year after the operation between the two groups (P>0.05; Table 7).

Discussion

BA is one of the main causes of neonatal obstructive jaundice and is the most common serious hepatobiliary disease in children. The main purpose of BA treatment is

Table 5 Recovery of liver function in two patient groups

	AST (U/L)	ALT (U/L)	γ-GT (U/L)	ALP (U/L)	TBA (μmol/L)
1st day after admission					
Control group (n=22)	177.84±21.26	173.53±19.87	513.56±61.34	754.87±98.65	119.26±27.87
Experimental group (n=25)	175.63±25.33	171.24±20.16	511.87±65.01	703.42±112.43	116.83±29.48
χ^2/t	0.3214	0.3911	0.0913	1.6569	0.2892
P	0.7493	0.6975	0.9276	0.1045	0.7737
7 days after the operation					
Control group (n=22)	134.25±16.79	148.64±17.94	468.54±38.97	648.97±65.21	92.64±11.25
Experimental group (n=25)	122.34±15.38	132.58±14.86	431.89±31.28	604.52±55.28	84.71±9.67
χ^2/t	2.5379	3.3561	3.5740	2.5293	2.5991
P	**0.0146**	**0.0016**	**0.0008**	**0.0150**	**0.0126**
30 days after the operation					
Control group (n=22）	108.24±14.26	112.74±9.68	416.84±35.24	508.94±51.67	83.14±9.64
Experimental group (n=25)	91.67±10.43	97.95±8.43	395.87±30.48	476.81±43.93	72.97±8.13
χ^2/t	4.5838	5.5998	2.1879	2.3043	4.1556
P	**<0.0001**	**<0.0001**	**0.0339**	**0.0258**	**0.0001**
60 days after the operation					
Control group (n=22)	90.44±9.68	96.34±8.59	382.68±29.67	469.64±48.24	70.36±8.84
Experimental group (n=25)	76.95±8.28	70.94±8.22	305.69±23.88	319.57±61.58	53.49±6.97
χ^2t	5.1500	10.3505	9.8498	9.2078	7.3069
P	**<0.0001**	**<0.0001**	**< 0.0001**	**<0.0001**	**<0.0001**

AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GT: γ-glutamyltransferase; ALP: alkaline phosphatase; TBA: total bile acid to improve the obstruction through early surgery.[16] Surgery is followed by the use of antibiotics and steroids to prolong the survival of children and reduce the occurrence of postoperative complications.[17] Most children with BA are malnourished and have growth retardation. Parenteral and enteral nutrition can significantly improve the nutritional status and immune function of patients after surgery, however, enteral nutrition is a more economical approach. Enteral nutrition helps to maintain the integrity of the structure and function of the intestinal mucosa and effectively reduces bacterial translocation and enterogenous infection to accelerate portal vein blood circulation.[18,19] These changes shorten the length of hospital stays and reduce complications following. The role of enteral nutrition in promoting postoperative recovery has been verified in infant digestive tract reconstruction.[20] Based on the *Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Children Critically Ill* Patient in the United States, enteral feeding should be initiated within 24-48 hours after the operation without waiting for anal exsufflation or defecation.[21] Therefore, EEN is of particular significance in children following gastrointestinal surgery.

This study investigated the effect of EEN on children with BA after the Kasai operation. We showed that the growth and nutritional status of children in the EEN group were significantly better compared to the TPN group on the 30th and 60th days after the operation. The levels of TBil and DBil in the EEN group were significantly lower than in the TPN group on the 30th and 60th days after the operation. Our results suggest that EEN can effectively improve the nutritional status of children and promote the subsidence of jaundice.

The improper treatment of jaundice in children can allow it to persist for a long time and serum bilirubin can pass through the underdeveloped blood-brain barrier. Under the influence of high-risk factors such as infection, acidosis and hypoxia, this can cause damage to the central nervous system resulting in high rates of mortality and can impact the intellectual development of children.[22,23] On the 7th, 30th and 60th days after the operation, the levels of AST, ALT, γ-GT, ALP and TBA were lower than on the first day after admission. The indices in the EEN group were lower than the TEN group suggesting that EEN intervention can improve liver function more than TEN intervention.

The pathological process of BA is characterised by progressive and occlusive lesions of the bile duct which can affect liver function in children.[24] We showed that before recovery with oral feeding, the number of *Bifidobacterium* and *Lactobacillus* in the feces of children in the EEN group was higher than the TPN group. The number of *Enterococcus* and *Escherichia coli* in the feces of children in the EEN group was lower than in the TPN group.

The balance of intestinal microecology plays a very important role in the growth and development of infants. A few hours after birth, in the primary succession of flora,

Table 6 The bacterial counts detected by plate culture

	***Bifidobacterium* (logN/g)**	***Lactobacillus* (logN/g)**	***Enterococcus* (logN/g)**	***Escherichia coli* (logN/g)**
	Before operation			
Control group（n=22）	2.34±0.58	2.48±0.68	4.55±0.64	5.14±0.81
Experimental group（n=25）	2.35±0.63	2.51±0.62	4.48±0.73	5.07±0.92
χ2/t	0.05634	0.1582	0.3473	0.2751
P	0.9553	0.8750	0.7299	0.7845
	1st day after the operation			
Control group（n=22）	3.67±0.69	3.09±0.55	4.07±0.63	4.84±0.36
Experimental group（n=25）	3.81±0.53	3.43±0.61	3.89±0.50	4.69±0.32
χ2/t	0.7852	1.9958	1.0909	1.5125
P	0.4364	0.0520	0.2811	0.1374
	Before oral feeding			
Control group（n=22）	4.59±0.46	3.88±0.39	3.75±0.42	4.19±0.37
Experimental group（n=25）	5.01±0.57	4.06±0.28	3.51±0.30	3.97±0.26
χ2/t	2.7547	2.0231	2.2743	2.3806
P	**0.0084**	**0.0490**	**0.0278**	**0.0216**

gastrointestinal bacteria appear. After physiological and secondary succession, the flora reaches a peak at the community stage. From late infancy to weaning, the structure of the intestinal flora tends to be stable and is maintained during childhood and in young adults.[25,26] Urao et al[27] found that after 2 weeks of probiotic treatment, the proportion of bifidobacteria in the intestinal tract of children with BA increased significantly, whilst the proportion of *Escherichia coli*, *Streptococcus* and other potential pathogens and endotoxin levels decreased. These findings suggest that probiotics can improve the intestinal flora of children with BA and reduce inflammatory reactions. The gut consists of around 10^{14} kinds of bacteria, fungi and microorganisms that play an important role in human intestinal health. The intestinal microbiota provides energy and nutrition to intestinal epithelial cells by producing short chain fatty acids.[28] It can also promote intestinal immunity by acting on the Toll like receptor.[29] Conversely, when the microbiome is imbalanced, systemic infection can occur.[30]

Bifidobacteriaceae, such as *Bifidobacterium longum* and *Bifidobacterium adolescentis*, are abundant anaerobic bacteria in the microbiota of healthy infants.[31] These bacteria degrade complex polysaccharides into

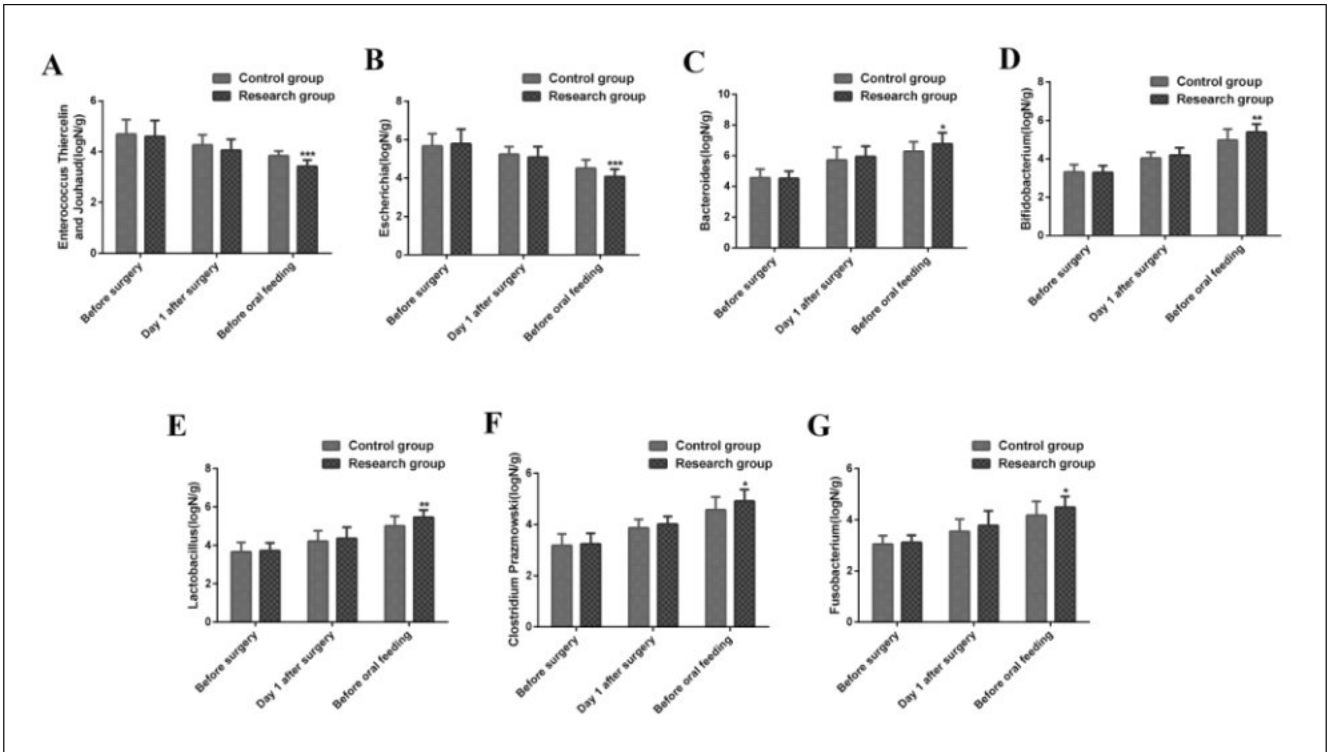

Figure 2 Results from the fluorescence quantitative sample analysis (*A) Enterococcus Thiercelin and Jouhaud; (B) Escherichia; (C) Bacteroides; (D) Bifidobacterium; (E) Lactobacillus; (F) Clostridium Prazmowski; (G) Fusobacterium; Compared with control group, *P<0.05, **P<0.01, ***P<0.001*

Table 7 Follow-up outcomes of the two groups at 12 months after the operation

	Number of cases of cholangitis (%)	The first time of the initial cholangitis (month)	The attack frequency (time)	Survival rate of autologous liver
Control group (n=22)	10 (45.5)	3.54±1.67	2.38±0.67	17 (77.3)
Experimental group (n=25)	8 (32.0)	5.43±1.45	1.56±0.49	21 (84.0)
χ^2/t	0.8965	2.5258	2.8910	0.3421
P	0.3437	0.0225	0.0106	0.5586

intermediates of short chain fatty acids such as acetate and lactate.[32] Finally, after a one-year follow-up, the incidence of cholangitis and the survival of autologous liver showed that the average time before the first incident of postoperative cholangitis in the EEN group was longer than in the TPN group. The average attack frequency in the EEN group was lower than in the TPN group. Most pathogenic agents of cholangitis after BA are intestinal flora, the most common of which are Gram-negative bacilli, such as *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.[33] Our data suggest that EEN may reduce the incidence of cholangitis by improving the balance of gastrointestinal flora.

Our study provides robust findings yet has a number of limitations. The sample size of the patients in this study was small and our findings required further validation in a larger patient cohort. The effect of cholangitis prevention and yellowing is an important indicator of postoperative efficacy. Also, the Kasai operation for biliary atresia does not represent the end of treatment. Postoperative treatment and long-term follow-up are required for children with biliary atresia. Follow-up plans and should be established and timely preventive measures should be given during follow-up visits. Consequently, a well-designed, larger study with long-term follow up study is needed to validate our findings.

In conclusion, EEN can improve the nutritional status of children with biliary atresia after the Kasai operation and promotes the subsidence of jaundice and the recovery of liver function. This approach may reduce the frequency of cholangitis by improving intestinal flora and so the use of early enteral nutrition should be considered in the clinic.

Acknowledgments

Not applicable.

Funding

This research was supported by the paediatric nutrition support team developing scientific research projects (No. xm-2019-000-0114-03) and the Guangdong Medical Research Fund (No. A2019541) and the Sanming Project of Medicine in Shenzhen (No. SZSM201812055).

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patient Consent for Publication

Informed consent was obtained from all individual participants included in the study.

Competing Interests

The authors declare no competing financial interest.

Authors' Contributions

Zhouguang Wu, Wenjie Zhang conceived and designed the experiments. Zhen Cheng, Siqi Chen selected the literature, extracted data and analysed it. Taoyan Zuo, Jingru Fu, Bin Wang, wrote the manuscript.

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