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Hepatic Expression of Transforming Growth Factor Beta 1 (TGFβ1) in Infants with Biliary Atresia

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ABSTRACT

Background: In advanced biliary atresia, the majority of TGF-hepatic expression is restricted to hepatocytes. These results imply paracrine fibrogenesis-driven by TGF-β1 in advanced biliary atresia.

Objective: To study the hepatic expression of TGF-β1 in biliary atresia (BA) as well as other causes of neonatal cholestasis. **Patients and Methods:** This study included sixty infants from those who attended to the Pediatric Hepatology Gastroenterology and Nutrition Department, National Liver Institute, Menoufia University from September 2019 to May 2021. They were divided into two groups: Group 1 (biliary atresia group): included 30 infants diagnosed as biliary atresia patients that was subdivided into 2 sub-groups, successful Kasai group (14) and failed Kasai group (16). Group 2 (non-biliary atresia cholestatic group): included 30 infants with other causes of neonatal cholestasis as control group. **Results:** Patients with BA were easily distinguished from those with other infantile cholestatic liver disorders using the BA scoring system (98.83 percent). There was significant difference between the successful and failed Kasai groups regarding postoperative complication, recurrent cholangitis, surgical outcome at (three months, six months, one year, two years postoperatively), ($P < 0.05$). Hepatic TGF Beta1 expression (in both biliary epithelial cells and liver cells) differed significantly between the non-atresia cholestatic group and both BA subgroups ($P < 0.01$).

Conclusions: Intraoperative cholangiography (IOC) verified the diagnosis of BA in surgical patients who were promptly referred for treatment. Hepatic expression of TGF Beta1 (biliary epithelial cells and liver cell expression) was significantly different between the non-atresia cholestatic group and biliary atresia subgroups.

Keywords: Transforming Growth Factor Beta 1 (TGFβ1), Infants, Biliary atresia.

INTRODUCTION

The extra-hepatic biliary ducts (EHBDs) and the intra-hepatic biliary system (IHBDs) are both damaged by biliary atresia (BA), a pediatrics idiopathic obstructive cholangiopathy, but the former is more seriously harmed. Hepatosplenomegaly, chronic jaundice, clay-colored stools, and dark urine are typical signs that will eventually appear soon after birth. In most cases, it is characterized by increasing inflammation and fibrosis that results in secondary biliary cirrhosis [(1)]. Although the precise cause of BA is unknown, it is generally accepted that the BA phenotype can be caused by a variety of different causes of liver damage, eliciting a stereotypical response that includes inflammation, bile duct growth, apoptosis, and fibrogenesis [(2)].- Most researchers have concentrated their study on a small number of inflammatory factors, such as tumour necrosis factor (TNF), interleukin (IL)-2, IL-8, IL-12, and IL-18. As well as transforming growth factor beta1 (TGF-β1) [(2)].

An accelerated portoenterostomy procedure and prompt diagnosis are essential components of managing biliary atresia (BA). Surgeries like these may seem promising at first, but only about 20% of seriously ill children actually benefit from them in the long run. Because of complications from cirrhosis, such as portal hypertension and stunted growth, the majority of affected children need to undergo liver transplantation (LT) to increase their chances of survival [(3)].

The profibrogenic, anti-inflammatory, and immunosuppressive actions of TGF-β protein are many. The proper coordination of these activities is necessary to maintain tissue homeostasis, and an abnormal expression of TGF-β is connected to several liver disease processes [(3)].In advanced biliary atresia, the majority of TGF-hepatic expression is restricted to hepatocytes. In advanced biliary atresia, findings point to paracrine pathways of TGF-1-driven fibrogenesis. As a result, some researchers advise using its blood level assessment as a diagnostic tool for biliary atresia, while others suggest using new developments in cell biology to target TGF-β activity or production inhibition as a major target for the development of antifibrotic therapies [(2)]. The purpose of this research was to study the hepatic expression of TGF-β1 in biliary atresia and other causes of neonatal cholestasis.

PATIENTS AND METHODS

The study was carried out at Menoufia University's National Liver Institute in the Pediatric Hepatology, Gastroenterology, and Nutrition Department. The research took place from September 2019 to May 2021.

Type of the study: Our study was retrospective study.
Subjects: Subjects included in the study were 2 groups:
Group 1 (biliary atresia group): included 30 infants diagnosed as biliary atresia patients by intraoperative cholangiogram.
Group 2 (non-biliary atresia cholestatic group): included 30 infants with other causes of neonatal cholestasis as control group.

Inclusion criteria: Infants diagnosed as biliary atresia.

- Age matched other causes of neonatal cholestasis.

Exclusion criteria

- Neglected biliary atresia.

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- Infants with comorbidities (congenital diseases, other genetic disorders, other organic diseases such as cardiac, renal, thyroid diseases, and gastro-intestinal diseases).

Methodology:

1. Full history taking
2. Thorough clinical examination: stress was laid on: jaundice, color of urine and stools, anthropometric measurements, hepatomegaly, splenomegaly, ascites, dysmorphic features and congenital anomalies.
3. Ophthalmological examination: For all infants for cataract, posterior embryotoxins, optic disc drusen and chorioretinitis done by ophthalmologist.
4. Complete blood count.
5. Liver function tests (total and direct serum bilirubin, total protein, albumin, ALT, AST, ALP, and GGT).
6. Prothrombin time and concentration.
7. TORCH screening including toxoplasmosis, rubella, herpes simplex viruses one and two and cytomegalovirus antibodies.
8. Abdominal ultrasonography.
9. Needle liver biopsy for all studied patients for routine pathological assessment.
10. Hepatic expression of transforming growth factor beta by immunohistochemical staining for BA atresia group were done on the intraoperative liver biopsy samples, but that for non-biliary atresia cholestasis group were done on the needle liver biopsy routinely done for diagnosis.

Ethical approval:

This experiment was ethically approved by the Menoufia University's (IRB Approval No. NLI IRB 00003413). After being fully informed, all the caregivers of the participants provided written consent. The study was conducted out in line with the Helsinki Declaration.

Statistical Analysis

The data were analyzed using a computer program called Statistical Package for the Social Sciences (SPSS), version 20. The results were presented using graphical and tabular formats. Mean, standard deviation, and confidence intervals were displayed for the numerical data. Qualitative data were presented as frequency and percentage. When evaluating data containing numerical independent variables, the student's t test (T) was typically utilised. Quantitatively distinct data were evaluated using Pearson Chi-Square. P values of 0.05 or below were deemed statistically significant.

RESULTS

Cases in this study were involved in two main groups namely: non-atresia cholestatic group (30 infants, 50%) and biliary atresia (BA) group who underwent Kasai operation (30 infants, 50%). The outcome of BA group after surgery was evaluated by the clearance of jaundice (S. bilirubin ≤ 2 mg/dL) within 6 months postoperatively. BA infants were subdivided into successful Kasai operation group (14 infants, 23%) and failed Kasai operation group (16 infants, 27%). Table (1) shows that there was statistically significant difference regarding male gender between non-atresia cholestatic group (66.7%) and all BA group (40%). The non-atresia cholestatic group was significantly older than BA group at presentation and also non-atresia cholestatic group was significantly older than the BA group at time of biopsy at presentation. There was statistically significant difference between successful Kasi and failed Kasi group age at time of biopsy at presentation, but no significant difference between both at the time of surgery and intraoperative biopsy.

Table (1): Demographic data among the studied groups

Variables	Non-atresia cholestatic group (N=30)	BA group (N=30)	BA group		P	P^0
			Successful Kasai (N=14)	Failed Kasai (N=16)		
Male Sex n (%)	20 (66.7)	12 (40)	6 (42.86)	6 (37.5)	0.112	0.038*
	Mean ± SD		**Mean ± SD**	**Mean ± SD**		
Age at presentation (days)	87.8±88.26	41±15.65	37.86±14.05	43.75±16.90	0.023*	0.006*
P^1=0.043, P^2=0.055, P^3=0.32						
Age of Biopsy at presentation (days)	111.23± 89.36	54.069±18.87	51.14± 12.57	56.8± 15.41 (N=15)	0.006*	0.001*
P^1=0.017, P^2=0.025, P^3=0.006*						
Age at Surgery (days)	--------	73.43± 18.87	70.64± 15.99	75.88± 21.29	0.458	--------
Current age (months)	33.36± 18.33	43.4± 14.65	43.00± 15.80	43.75± 14.08	0.086	0.026*

SD: Standard deviation, ***significant**

P: Comparison between non-atresia cholestatic group, Successful Kasai and Failed Kasai, P^0: Comparison between BA group and non-atresia cholestatic group, P^1: Comparison between non-atresia cholestatic group and Successful Kasai. P^2: Comparison between non-atresia cholestatic group and Failed Kasai, P^3: Comparison between Successful Kasai group and Failed Kasai.

Table (2) shows that, PLT were significantly higher among all BA group than non-atresia cholestatic group, especially higher in successful Kasai group. ALT was significantly lower among all BA infants than non-atresia cholestatic group and significantly lower among failed Kasai group. Also, GGT was significantly increased among all BA infants and significantly increased among failed Kasai group than non-atresia cholestatic group. Total protein was significantly higher among non-atresia cholestatic infants than all BA infants; while there was no statistically significant difference between successful and failed Kasai subgroups as regards preoperative CBC parameters, CRP, and LFTs at presentation.

Table (2): Analysis of the preoperative CBC parameters, LFTs and CRP among the studied groups

Variable	Non-atresia cholestatic group (N=30) Mean ± SD	BA group (N=30) Mean ± SD	BA group		P	P^0
			Successful Kasai (N=14) Mean ± SD	Failed Kasai (N=16) Mean ± SD		
Hb (g/dl)	10.1± 1.2	9.9± 1.2	9.9± 1.3	9.9± 1.1	0.86	0.59
TLC (10^3 /µL)	13.6± 3.2	14.7± 3.5	14.9± 3.1	14.4± 3.4	0.76	0.49
PLT (10^3 /µL)	389.8±94.9	478.1± 113.7	546.4± 131.6	418.4± 92.8	0.01*	0.04*
P^1=0.002, P^2=0.55, P^3=0.07						
Retics %	2.4±0.5	3.1±0.6	2.9± 0.6	3.3± 0.7	0.21	0.1
CRP (mg/L)	11.6± 2.7	12.6± 3.1	11.4± 2.4	13.8± 3.4	0.89	0.79
TBIL (mg/dL)	12.4± 3.1	10.5± 2.2	10.3± 2.2	10.7±2.4	0.44	0.2
DBIL (mg/dL)	8.4± 1.5	7.5± 1.1	7.4± 1.3	7.5±1.6	0.73	0.43
Total Protein (µg/mL)	5.8± 1.03	5.2± 0.9	5.2± 0.8	5.2±1.1	0.08	0.02*
Albumin (g/dL)	3.8± 0.6	3.7± 0.5	3.8± 0.4	3.7±0.6	0.86	0.59
AST(U/L)	333.3± 28.3	262.2± 53.8	246.2± 51.7	276.2±59.2	0.25	0.12
ALT(U/L)	299.9± 40.1	166.6± 9.9	167.8± 7.9	165.6±8.7	0.02*	0.005*
P^1=0.05, P^2=0.04, P^3=0.94						
ALP(U/L)	651.1± 54.3	574.9± 43.6	581.6± 42.3	568.9±33.5	0.71	0.41
GGT(U/L)	455.2± 11.3	827.5± 21.6	683.3± 63.4	953.7±33.5	0.007*	0.005*
P^1=0.11, P^2=0.004, P^3=0.16						

CBC: Complete blood count, **CRP:** C-reactive protein, **LFTs:** Liver function tests, **Hb:** Hemoglobin **TLC:** Total leucocyte count, **PLT:** Platelets, **Retics P:** Reticulocyte count, **CRP:** C-reactive protein, **TBIL:** Total bilirubin, **D BIL:** Direct bilirubin. **AST:** Aspartate aminotransferase, **ALT**: Alanine transaminase, **ALP:** Alkaline phosphatase**, GGT:** Gamma-glutamyl transferase.

SD: Standard deviation, *Significant
P: Comparison between non-atresia cholestatic group, Successful Kasai and Failed Kasai
P^0: Comparison between BA group and non-atresia cholestatic group
P^1: Comparison between non-atresia cholestatic group and Successful Kasai
P^2: Comparison between non-atresia cholestatic group and Failed Kasai
P^3: Comparison between Successful Kasai group and Failed Kasai

Table (3) shows that, postoperative reticulocyte counts at one month follow up was significantly increased in all BA group than non-atresia cholestatic group, and significantly increased among infants with failed Kasai. Also, there was no statistically significant difference between all BA group and non-atresia cholestatic group regarding postoperative DBIL, but it was significantly lower among successful Kasai group than failed Kasai groups, and non-atresia cholestatic group; while, ALT was significantly higher among non-atresia cholestatic group than all BA infants, and significantly lower in successful Kasai group. Also, postoperative GGT at one month follow up was significantly lower among non-atresia cholestatic group than all BA infants, without statistical difference between successful Kasai and failed Kasai groups. On the other hand, there was no a statistically significant difference regarding other postoperative parameters of CBC, LFTs and CRP among the studied groups.

Table (3): Comparison among the studied groups regarding postoperative CBC, CRP and LFTs at 1 month follow-up

Variable	Non-atresia cholestatic group (N=30) Mean ± SD	BA group (N=30) Mean ± SD	BA group		P	P^0
			Successful Kasai (N=14) Mean ± SD	Failed Kasai (N=16) Mean ± SD		
Hb (g/dl)	10.47±1.20	9.64± 0.98	9.51± 1.03	9.76± 0.96	0.305	0.15
TLC (10^3 /µL)	13.21±3.1	13.80± 3.3	15.14± 3.6	12.64± 3.1	0.260	0.603
PLT (10^3 /µL)	426.57±98.6	447.53± 101.7	463.07±114.3	433.94± 101. 4	0.784	0.617
Retics %	2.24±0.4	2.97± 0.5	2.70± 0.5	3.21± 0.7	0.045*	0.026*
P^1=0.198, P^2=0.018, P^3=0.316						
CRP (mg/L)	7.13±1.1	12.91± 3.1	8.03± 1.8	17.18± 4.1	0.099	0.15
TBIL (mg/dL)	12.17±2.8	7.14± 1.5	5.34± 1.1	8.71±1.8	0.160	0.083
DBIL (mg/dL)	6.16±1.4	5.16± 1.5	3.56± 0.6	6.56±1.3	0.027*	0.263
P^1=0.026, P^2=0.715, P^3=0.002						
Total Protein (µg/mL)	5.73±0.83	5.41± 0.99	5.44± 1.05	5.39±0.98	0.413	0.18
Albumin (g/dL)	3.79±0.60	3.73± 0.42	3.89± 0.31	3.59±0.48	0.276	0.623
AST(U/L)	252.87±61.3	178.17± 42.3	149.9± 35.1	202.94±48.3	0.162	0.088
ALT(U/L)	217.27±51.3	140.36± 33.1	124.3± 28.6	154.44±34.7	0.022*	0.008*
P^1=0.020, P^2=0.093, P^3=0.229						
ALP(U/L)	573.80±41.2	518.06± 28.6	509.7± 122.3	525.38±130.1	0.735	0.44
GGT(U/L)	364.00±8.6	803.7± 97.3	891.4± 218.2	726.94±79.3	0.002*	0.001*
P^1=0.002, P^2=0.010, P^3=0.402						

CBC: Complete blood count, **CRP:** C-reactive protein, **LFTs:** Liver function tests, **Hb:** Hemoglobin **TLC:** Total leucocyte count, **PLT:** Platelets, **Retics P:** Reticulocyte count, **CRP:** C-reactive protein, **TBIL:** Total bilirubin, **D BIL:** Direct bilirubin. **AST:** Aspartate aminotransferase, **ALT**: Alanine transaminase, **ALP:** Alkaline phosphatase**, GGT:** Gamma-glutamyl transferase

SD: Standard deviation, *Significant
P: Comparison between non-atresia cholestatic group, Successful Kasai and Failed Kasai
P^0: Comparison between BA group and non-atresia cholestatic group
P^1: Comparison between non-atresia cholestatic group and Successful Kasai
P^2: Comparison between non-atresia cholestatic group and Failed Kasai
P^3: Comparison between Successful Kasai group and Failed Kasai

Table (4) shows that, postoperative reticulocyte count at 3 months follow-up was significantly increased in in all BA infants than non-atresia cholestatic group, and significantly increased among infant with failed Kasai. TBIL and DBIL were significantly lower among successful Kasai group than failed Kasai groups non-atresia cholestatic. Albumin was significantly lower among failed Kasai group than successful Kasai and non-atresia cholestatic groups. Also, postoperative GGT at 3 months follow up was significantly lower among non-atresia cholestatic than all BA infants, without statistical difference between failed Kasai and successful Kasai groups. On the other hand, there was no a statistically significant difference regarding other postoperative parameters of CBC, LFTs and CRP after 3 months follow-up among the studied groups.

Table (4): Comparison among the studied groups regarding postoperative CBC, CRP and LFTs at 3 months follow-up

Variable	Non-atresia cholestatic group (N=30) Mean ± SD	BA group (N=30) Mean ± SD	BA group		P	P^0
			Successful Kasai (N=14) Mean ± SD	Failed Kasai (N=16) Mean ± SD		
Hb (g/dl)	10.2±1.05	10.2±1.15	10.6± 1.2	9.8± 1.01	0.19	0.93
TLC (10^3 /µL)	13.1±3.1	11.9±2.7	12.3± 2.8	11.5± 2.5	0.45	0.25
PLT (10^3 /µL)	385.7±91.6	379.7±88.5	401.9±97.3	360.3± 87.1	0.71	0.87
Retics %	2.2±0.4	2.9±0.6	2.7± 0.6	3.07± 0.8	0.03	0.02*
P^1=0.12, P^2=0.016, P^3=0.38						
CRP (mg/L)	6.4±1.4	17.3±3.9	7.6± 1.6	25.9± 6.2	0.08	0.15
TBIL (mg/dL)	6.03±1.3	6.2±1.4	2.1± 0.4	9.8±2.3	0.001*	0.87
P^1=0.007, P^2=0.013, P^3<0.001						
DBIL (mg/dL)	4.25±1.8	4.4±1.1	1.2± 0.4	7.3±1.3	0.001*	0.86
P^1=0.006, P^2=0.014, P^3<0.001						
Total Protein (µg/mL)	5.5±0.8	5.6±1.1	5.9± 1.2	5.4±1.1	0.3	0.53
Albumin (g/dL)	4.02±0.6	3.8±0.5	4.1± 0.3	3.5±0.4	0.001*	0.09
P^1=0 .55, P^2=0.002, P^3=0.001						
AST(U/L)	200.3±48.3	170.5±38.3	128.5± 30.6	207.2±50.1	0.39	0.52
ALT(U/L)	162.3±37.2	153.4±36.3	112.1± 26.2	189.5±5.4	0.29	0.79
ALP(U/L)	511.4±113.5	472.7±116	394.2± 94.3	541.3±32.3	0.29	0.59
GGT(U/L)	284.6±68.1	654.4±60.1	563.3± 36.6	734.06±33.2	0.001*	<0.001*
P^1=0.01, P^2<0.001, P^3=0.18						

CBC: Complete blood count, **CRP:** C-reactive protein, **LFTs:** Liver function tests, **Hb:** Hemoglobin **TLC:** Total leucocyte count, **PLT:** Platelets, **Retics P:** Reticulocyte count, **CRP:** C-reactive protein, **TBIL:** Total bilirubin, **D BIL:** Direct bilirubin. **AST:** Aspartate aminotransferase, **ALT**: Alanine transaminase, **ALP:** Alkaline phosphatase**, GGT:** Gamma-glutamyl transferase.

SD: Standard deviation, *Significant
P: Comparison between non-atresia cholestatic group, Successful Kasai and Failed Kasai
P^0: Comparison between BA group and non-atresia cholestatic group
P^1: Comparison between non-atresia cholestatic group and Successful Kasai
P^2: Comparison between non-atresia cholestatic group and Failed Kasai
P^3: Comparison between Successful Kasai group and Failed Kasai

Table (5) shows no statistically significant difference among all BA infants and non-atresia cholestatic group regarding postoperative CBC parameter, except mean level of Hb, which was significantly lower among failed Kasai group than successful Kasai and non-atresia cholestatic groups. CRP was significantly increased among failed Kasai group than non-atresia cholestatic. Postoperative TBIL and DBIL at 6 months follow up were significantly increased among failed Kasai group than non-atresia cholestatic and successful Kasai groups; while, albumin was significantly higher among non-atresia cholestatic infants than all BA infants with statistically significant decreased level in failed Kasai group than successful Kasai groups. Also, postoperative GGT at 6 months follow up was significantly lower among non-atresia cholestatic infants than all BA infants, without statistical difference between successful and failed Kasai groups. On the other hand, there was no a statistically significant difference regarding other postoperative parameters of CBC, LFTs and CRP after 6 months follow-up among the studied groups.

Table (5): Comparison among studied groups regarding postoperative CBC, CRP and LFTs at 6 months follow-up

Variable	Non-atresia cholestatic group (N=30) Mean ± SD	BA group (N=30) Mean ± SD	BA group		P	P^0
			Successful Kasai (N=14) Mean ± SD	Failed Kasai (N=16) Mean ± SD		
Hb (g/dl)	10.15±1.23	9.38±1.27	10.44± 1.18	9.29± 1.12	0.023	0.321
P^1=0.466, P^2= 0.025, P^3= 0.011						
TLC (10^3 /µL)	12.82±3.1	12.18±2.8	12.29± 2.5	12.09± 2.7	0.858	0.587
PLT (10^3 /µL)	388.83±95.6	366.46±88.3	437.5± 101.3	304.3± 73.7	0.105	0.628
Retics %	2.29±0.4	2.7±0.5	2.50± 0.4	2.92± 0.5	0.149	0.108
CRP (mg/L)	5.02±1.04	19.73±4.2	8.23± 2.00	29.79± 7.2	0.042	0.084
P^1=0.315, P^2=0.028, P^3=0.197						
TBIL (mg/dL)	2.46±0.3	6.43±1.1	0.76± 0.11	11.41±2.3	0.001*	0.005*
P1=0.035, P2<0.001, P3<0.001						
DBIL (mg/dL)	1.7±0.4	4.38±1.01	0.36± 0.07	7.90±1.4	0.001*	0.01*
P^1=0.038, P^2<0.001, P^3<0.001						
Total Protein (µg/mL)	5.48±0.95	5.86±1.19	6.24± 0.90	5.54±1.33	0.083	0.17
Albumin (g/dL)	4.06±0.56	3.69±0.74	4.25± 0.43	3.21±0.62	0.001*	0.04*
P^1=0.264, P^2<0.001, P^3<0.0001						
AST(U/L)	127.37±28.5	156.66±37.2	128.9± 27.5	181.00±42.6	0.233	0.289
ALT(U/L)	113.17±26.3	113.30±27.1	95.79± 21.3	128.63±30.3	0.575	0.995
ALP(U/L)	376.1±70.3	474.36±116.4	346.4± 81.3	586.38±107.6	0.099	0.286
GGT(U/L)	231.57±55.3	605.24±107.3	431.2± 104.6	757.52±103.1	0.003*	0.004*
P^1=0.033, P^2=0.002, P^3=0.158						

CBC: Complete blood count, **CRP:** C-reactive protein, **LFTs:** Liver function tests, **Hb:** Hemoglobin **TLC:** Total leucocyte count, **PLT:** Platelets, **Retics P:** Reticulocyte count, **CRP:** C-reactive protein, **TBIL:** Total bilirubin, **D BIL:** Direct bilirubin. **AST:** Aspartate aminotransferase, **ALT**: Alanine transaminase, **ALP:** Alkaline phosphatase**, GGT:** Gamma-glutamyl transferase.

SD: Standard deviation, *Significant
P: Comparison between non-atresia cholestatic group, Successful Kasai and Failed Kasai
P^0: Comparison between BA group and non-atresia cholestatic group
P^1: Comparison between non-atresia cholestatic group and Successful Kasai
P^2: Comparison between non-atresia cholestatic group and Failed Kasai
P^3: Comparison between Successful Kasai group and Failed Kasai

Figure (1) shows that, BA score was significantly higher among all BA infants than non-atresia cholestatic infants (P<0.001), with statistically significant difference between failed Kasai group and successful Kasai group (P=0.024).

There was no statistically significant difference between failed Kasai group and successful Kasai group regarding surgical type of BA (P=0.024).

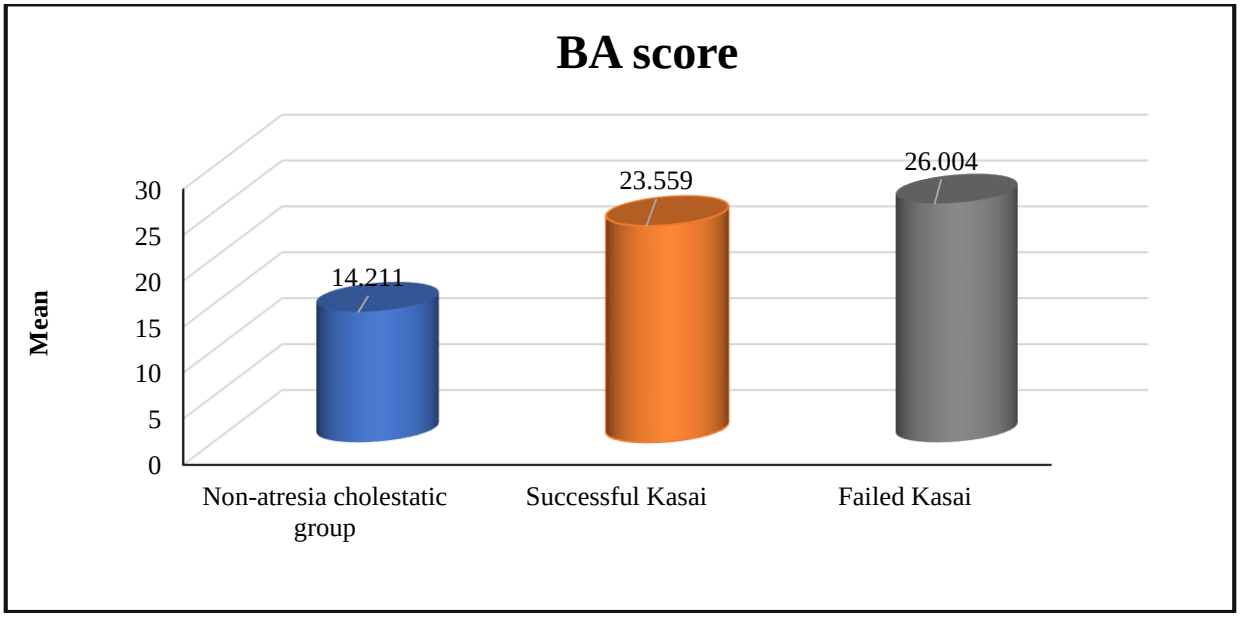

Figure (1): Biliary atresia (BA) score among the studied groups.

Figure (2) shows that, there was significant difference between the successful and failed Kasai groups regarding postoperative complication, recurrent cholangitis, surgical outcome (P <0.05).
On the other hand, there was no significant difference between the two groups regarding postoperative growth and outcome at (three years, five-year postoperatively).

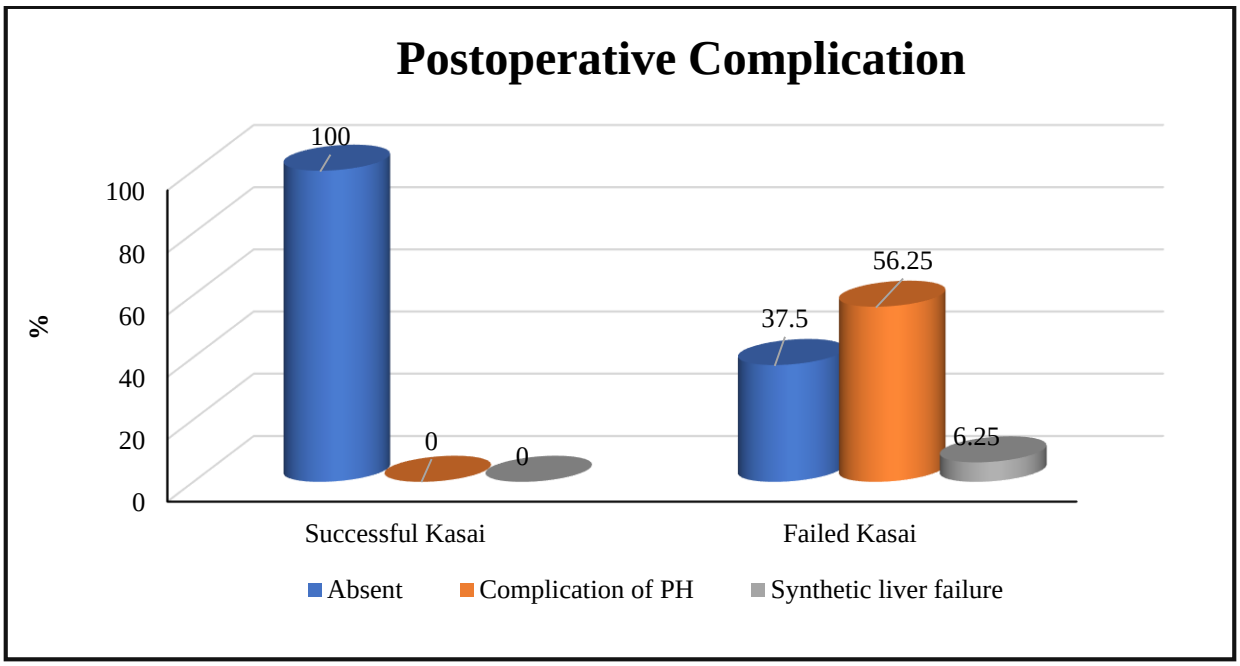

Figure (2): Comparison between successful and failed Kasai groups regarding postoperative complication

Table (6) shows that, there was statistically significant difference regarding the hepatic expression of TGF Beta[1] (biliary epithelial cells and liver cells expression) among non-atresia cholestatic group and both BA subgroups. However, with no statistically significant difference between successful Kasai and failed Kasai groups.

Table (6): Comparison among the studied groups regarding hepatic expression of TGF Beta[1]

Variable	Non-atresia cholestatic group (N=30) N (%)	BA group (N=30) N (%)	BA group		P	P^0
			Successful Kasai (N=14) N (%)	Failed Kasai (N=16) N (%)		
Hepatic expression, TGF Beta[1] biliary epithelial cells expression						
+1	11 (36.7)	15 (50)	9 (64.3)	6(37.5)	0.001*	<0.001*
+2	0 (0)	9 (30)	4 (28.6)	5(31.3)		
+3	0 (0)	2 (6.7)	1(7.1)	1(6.2)		
Negative	19(63.3)	4 (13.3)	0(0.0)	4(25)		
P^1=<0.001*, P^2=0.003*, P^3=0.204						
Hepatic expression TGF Beta[1] liver cells expression						
+1	16(53.3)	4 (13.3)	1(7.1)	3(18.8)	<0.001*	<0.001*
+2	7(23.3)	14 (46.7)	9(64.3)	5(31.3)		
+3	1(3.3)	12 (40)	4(28.6)	8(50)		
Negative	6(20)	0 (0)	0(0)	0(0)		
P^1<0.001*, P^2<0.001*, P^3=0.187						

TGF: Transforming Growth Factor, *Significant

P: Comparison between non-atresia cholestatic group, Successful Kasai and Failed Kasai

P^0: Comparison between BA group and non-atresia cholestatic group

P^1: Comparison between non-atresia cholestatic group and Successful Kasai

P^2: Comparison between non-atresia cholestatic group and Failed Kasai

P^3: Comparison between Successful Kasai group and Failed Kasai

Figures (3, 4) show that, there was statistically significant but weak positive correlation between GTT at presentation, and at one, three and six months postoperatively with hepatic expression of TGF Beta[1] from liver epithelial cells expression. Also, there was statistically significant but weak negative correlation between total serum bilirubin at presentation, however, there was statistically significant but weak positive correlation between total serum bilirubin at six months postoperatively with the hepatic expression of TGF Beta[1] from liver epithelial cells.

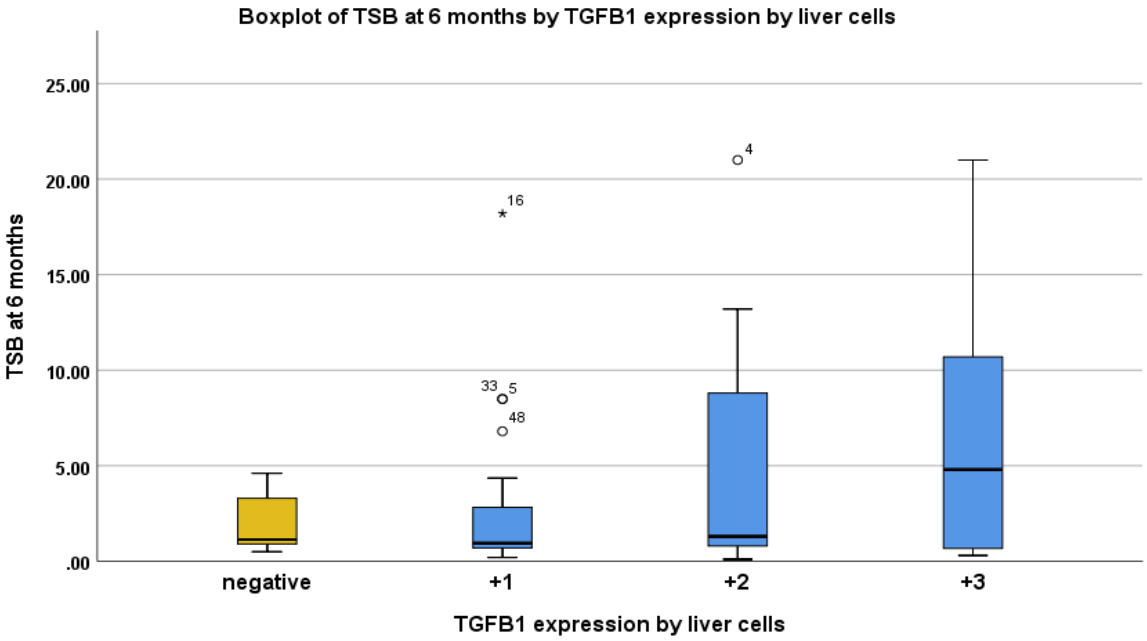

Figure (3): simple boxplot of total serum bilirubin at six months by hepatic expression of TGF Beta[1] by liver cells.

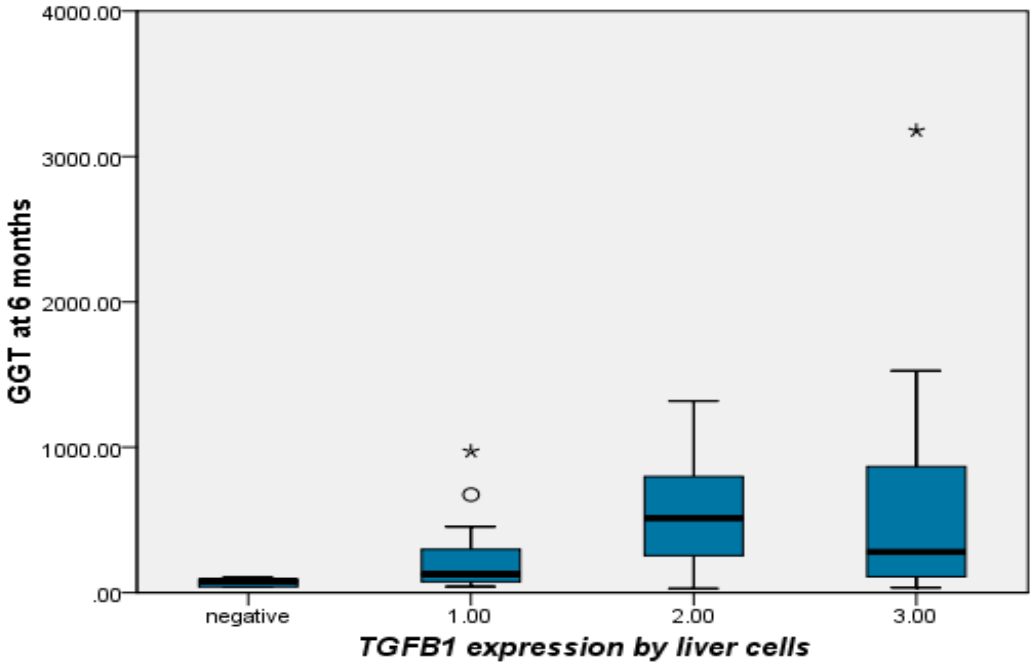

Figure (4): simple boxplot of total serum GGT at six months by hepatic expression of TGF Beta[1] by liver cells

Figure (5) shows that, there was statistically significant but weak positive correlation between GTT at presentation and after three months with biliary epithelial cells expression of TGF Beta[1].

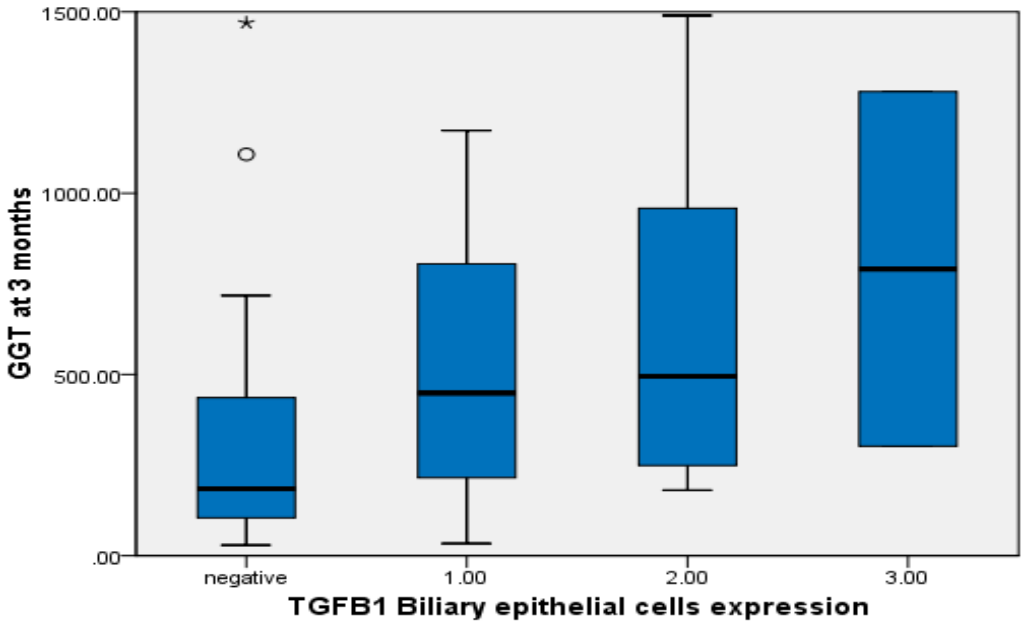

Figure (5): Simple boxplot of total serum GGT at three months by biliary expression of TGF Beta[1] by liver cells.

DISCUSSION

A key modulator of liver cell replication and proliferation, transforming growth factor-1 (TGF- β^1) is linked to liver fibrosis. Moreover, it is an inducer of hepatic stellate cells, the principal precursor in liver fibrogenesis **(4).**

The demographic data of the studied groups recorded in this study (Table 1), showed that percent of male gender was higher (66.7%) among non-atresia cholestatic group than all BA infants, with statistically significant difference (P= 0.038). Regarding the age at presentation, the non-atresia cholestatic group (87.8±88.26 days) was significantly older than BA group (41±15.65 days) at presentation (P = 0.006). This characteristic differs our study than other studies, for instance, **Kandil *et al.*** **(5)** reported that the BA group infants (68.88± 10.44 day) were older at presentation than cholestatic group (57.76± 16.66 day) infants; and **Ghoneim *et al.*(6)** also noted that the BA group infants (92.7 ± 55.5 day) were older at presentation than cholestatic group (84.4 ± 49.4 day) infants.

In the BA infants, the biopsies were taken twice, preoperatively on presentation and intraoperatively in the same day of the surgery (Table 1). The mean age of preoperative biopsy was significantly higher in non-atresia cholestatic group (111.23± 89.36 days) than all BA infants (54.069±18.87 days), (P= 0.001), this is because non-atresia cholestatic infants presented with older age. Our results agreed with **Sira *et al.*** **(7)** who reported the mean age of liver biopsy (72.5 ± 40.5 days) in BA infants and (110.6 ± 54.8 days) in non-BA infants. Our results are also comparable to the study conducted by **Kandil *et al.*** **(5)**, who found that all BA cases were less than 90 days old, and ranged between 44 and 89 days.

Our results are matching with the study by **Kandil *et al.*** **(5)** who found that the mean level of platelets in BA group (515.68 ± 185.947 IU/l) was significantly higher than that in non-BA group (347.63 ± 144.504 IU/l), (P <0.0001). Also, **Sira *et al.*** **(7)** reported that platelet count was significantly higher in patients with BA compared with other cholestatic disorders (P= 0.001) and this suggests that platelets in BA are activated and may have a role in the inflammatory process in BA.

Kandil *et al.* **(5)** was in line with our finding that total protein was higher among the cholestatic group than BA group but without statistically significant difference. In other hand, our study showed that, there was no a statistically significant difference between successful and failed Kasai groups regarding preoperative liver function parameters. In contrast to our result, **Kandil *et al.*** **(5)** mentioned that liver transaminases (AST and ALT) were significantly higher in BA compared with non-BA group, (P < 0.05).

In our study, we analyzed postoperative LFTs, CBC parameters and reticulocytic count at 1, 3, 6 months follow up, our results are in line with **Gad *et al.*** **(8)** study which noted that post-Kasai portoenterostomy (KPE) jaundice resolution was an independent predictor of long-term natural liver survival (NLS) as the level of TBIL and DBIL were significantly lower at 6 months postoperatively among infant with NLS.

We found that mean level of GGT at 1, 3, 6 months follow up was significantly lower among non-atresia cholestatic than all BA infants, without statistical difference between successful and failed Kasai groups (p<0.05). **Yassin *et al.*** **(9)** found that, postoperative AST

at 6 months follow up was significantly lower in the successful group. Other authors found that the preoperative AST was elevated significantly in all children with BA, 4 months postoperatively it declined significantly by 2-fold in the anicteric group [(10)].

About BA score, our study showed that BA score was significantly higher among all BA infants (24.86±3.013) than non-atresia cholestatic group (14.21± 3.09) (P<0.001), with statistically significant difference between failed Kasai group (26± 2.26) and successful Kasai group (23.56± 3.3) with (P=0.024). Our study agreed with **El-Guindi *et al.*** [(11)] who concluded that BA scoring system accurately separates infants with BA and those with other causes of neonatal cholestasis (NC) without the need of intraoperative cholangiography (IOC).

Our finding was also compatible with literatures published by **Decharun *et al.*** [(12)] who reported that cholangitis is one of the most common complications after Kasai surgery occurring in 40-93% of cases.

Our study showed that, there was statistically significant difference regarding the hepatic expression of TGF-β^1 (biliary epithelial cells and liver cells expression) between non-atresia cholestatic group and all BA infants (P<0.01), with no statistically significant difference between successful Kasai and failed Kasai groups, and the expression of TGF-β^1 was more advanced in BA group than non-atresia cholestatic group and also, in hepatocytes was stronger than in biliary epithelial cells. In contrast to our study, the study by **Li *et al.*** [(13)] revealed that the BA group in comparison to a group of neonatal hepatitis, TGF-β^1 were expressed in both hepatocytes and biliary epithelial cells, and the expression in biliary epithelial cells was stronger than in hepatocytes.To extent of our knowledge, our study is the first attempt to find the correlation of hepatic expression of TGF-β^1 with the liver function tests and also with the success of Kasai operation by jaundice clearance in BA infants. Our results suggested that hepatic expression of TGF-β^1 in intraoperative biopsy, may be used as a diagnostic parameter for BA infants from other non-BA causes of cholestasis as high hepatic expression of TGF-β^1 from liver and biliary epithelial cells was noticed in biopsies from BA infants.

CONCLUSION

In conclusion, by using the BA scoring system, we could clearly separate patients with BA and those with other infantile cholestatic liver diseases with high accuracy (98.83%). There was statistically significant difference regarding the hepatic expression of TGF Beta1 (biliary epithelial cells and liver cells expression) between non-atresia cholestatic group and biliary atresia subgroups. And there was no statistically significant difference between failed and successful Kasai groups. There was statistically significant but weak positive correlation between GTT at presentation, and at one, three and six months postoperatively with hepatic expression of TGF Beta1 from liver epithelial cells expression. And there was statistically significant positive correlation between BA score with hepatic expression of TGF Beta[1] from liver epithelial cells and biliary cells expression.

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