Aim: This study investigates the fall in total serum bilirubin levels within 1 week after surgery, as a marker to predict early outcome in biliary atresia (BA) patients post-Kasai portoenterostomy (KP).

Methods: The ratio of total serum bilirubin levels at the 7th postoperative day to the preoperative level (TB7/TB0) in patients undergoing KP was calculated (January 2011–July 2015). Patients were stratified after 3-months follow-up into outcome groups depending on the clinical clearance of jaundice and TB7/TB0 ratio was correlated to outcome and liver histopathological changes in these groups.

Results: Sixty-one patients (M:F = 44:17), median age 75 days were included. At the end of 3 months, 27 (44.39%) were anicteric while 26 (42.6%) were still clinically jaundiced. Patients with a higher median value of TB7/TB0, that is, 0.856 were more likely to have jaundice at the end of 3 months as compared to patients with a lower median value of 0.615 (P < 0.0001). A cutoff TB7/TB0 ratio >0.723 predicted the KP outcome with 84.6% sensitivity and 81.5% specificity. The difference in TB7/TB0 ratio between patients with varying severity of liver histopathological changes was also significant, namely, cholestasis (P = 0.01), hepatocellular damage (P = 0.03), portal inflammation (P = 0.04), and portal fibrosis (P = 0.02).

Conclusions: The rapidity of fall in the total serum bilirubin levels within 1 week post-KP was able to predict the likely outcome in BA patients.

Keywords: Biliary atresia, Kasai portoenterostomy, serum bilirubin, surgical outcome

INTRODUCTION

Biliary atresia (BA) is a rare but serious condition with an incidence of 1 in 10,000–1 in 16,700 live births where pathological jaundice persists beyond 2 weeks of life.[1,2] If left untreated, it has a relentless course to chronic liver disease with progressive obliteration of the intrahepatic biliary tree and liver cirrhosis with essentially no survivors by the age of 2 years.[3] Kasai portoenterostomy (KP) is the primary therapeutic option for establishing biliary drainage. About 25% of the total patients undergoing KP will be cured, and in the remaining patients, progressive liver failure develops that can be salvaged only by transplantation.[4-6]

Many studies have focused on the prognostic factors both biochemical and histopathological associated with the KP but it is still controversial as to which of these indicators will predict a successful or failed KP. These include bridging fibrosis, extensive liver fibrosis, direct bilirubin (DB) levels and aspartate aminotransferase levels 2 months after KP, gamma-glutamyl transferase, and bilirubin levels at 5 weeks after KP.[7-12]

The most pressing question after KP is whether the child is likely to be treated successfully leading to a cure or will progress to chronic liver disease and require liver transplantation (LT). Due to the lack of defined objective

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criteria, it is not possible to give an objective prognosis in the early postoperative period. This is particularly a difficult situation for the parents of these children.

A successful KP is defined by the clearance of clinical jaundice and a fall in the total serum bilirubin levels to normal value postoperatively. We hypothesized that the rapidity of fall in total serum bilirubin in 1 week should be able to predict the long-term outcome of KP.

This study was conducted to seek a simple ratio of serum total bilirubin at the 7th day postoperative to its preoperative levels (TB7/TB0) as a marker to predict early, the outcome of children with BA undergoing KP. A relationship between the severity of the histopathological changes in liver biopsy and the TB7/TB0 ratio was also studied.

**MATERIALS AND METHODS**

The study was approved by the Ethics Committee of All India Institute of Medical Sciences vide letter number IESC/T20/03.01.2014. All patients who were diagnosed with extrahepatic BA and who underwent KP during January 2011 to July 2015 were included. Patients whose parents did not give consent for the study, who were lost to follow-up or those who died before 3 months of follow-up were excluded from this study. The patients were evaluated clinically, biochemically, and radiologically at presentation. Data of age/gender, symptoms (discoloration of skin or sclera/clay colored or mixed stools/high-colored urine/abdominal distension), signs (icterus/hepatomegaly/splenomegaly/ascites), abdominal ultrasonography, hepatobiliary scan, biochemical liver function tests, and total serum bilirubin at the 7th postoperative day were collected. The TB7/TB0 ratio was calculated for each patient.

During KP, a wedge biopsy of the liver was taken and sent for histopathological evaluation. All the liver biopsy samples were stained with hematoxylin and eosin and were analyzed by a single senior hepatobiliary pathologist. The intraoperative wedge biopsy of the liver was histologically graded according to the histological scale as described previously.[13]

The patients were followed up for clinical disappearance of jaundice, the color of the stools, and the serum bilirubin levels. On this basis, they were stratified into three outcome assessment groups separately at the end of 3 months [Table 1].

| Jaundice | Group 1 | Group 2 | Group 3 |
|----------|---------|---------|---------|
| At 1 month | Disappeared | Disappeared | Persist |
| At 3 months | Disappeared | Present | Persist |

| Colour of stools | At 1 month | At 3 months | At 1 month | At 3 months |
|----------------|-----------|-----------|-----------|-----------|
| Yellow | Yellow | Clay | Yellow | Clay |

| Total serum bilirubin levels | At 1 month | At 3 months |
|-----------------------------|-----------|-----------|
| Normal | Normal | Elevated<sup>a</sup> |
| Elevated<sup>a</sup> | Elevated<sup>a</sup> |

<sup>a</sup> Total serum bilirubin more than 2 mg/dl were considered elevated

1 month was compared using McNemar’s test. The difference in median TB7/TB0 was compared with severity of histological changes using Kruskal–Wallis test. The difference in medians of TB7/TB0 was compared with the outcome using Wilcoxon rank-sum test. The predictive ability of TB7/TB0 was calculated for the clinical outcome using receiver operating characteristics (ROC) curves. The results are presented as cutoff, sensitivity, specificity, rate of correct classification, likelihood ratio, and area under the ROC curve (AUC), 95% confidence interval. The P < 0.05 was considered statistically significant.

**RESULTS**

A total of 61 patients, 44 (72.1%) males and 17 (27.9%) females who underwent KP for BA were included in this study; 5 died and 2 were lost to follow-up. Fifty eight patients were followed up till 1 month and 54 till 3 months postoperatively. The mean age of presentation was 83.9 ± 35.9 days, median 75 days with the youngest child 24 days old and the oldest 165 days.

All children presented with jaundice and high colored urine. All patients had hyperbilirubinemia with the mean bilirubin level of 11.6 ± 4.1 mg/dl and median of 10.1 mg/dl ranging from 6.2 to 25 mg/dl.

Liver histopathology revealed that all patients had cholestasis with Grade 2 cholestasis in most of them -44 (72.1%). Hepatocellular damage Grade 1 (ballooning degeneration) was present in 15 (24.6%), Grade 2 (fatty degeneration) in 38 (62.3%), and Grade 3 (necrosis) in 8 (13.1%) patients. Most liver biopsy specimen showed moderate bile duct proliferation-39 (63.9%). Almost equal proportions of patients had Grade 1 (mild), Grade 2 (moderate) and Grade 3 (severe) degrees of bile duct inflammation. All cases showed some degree of portal edema and portal inflammation. Most of the patients 46 (75.4%) had severe degree of fibrosis.
At the end of 1 and 3 months, 18 (31.0%) and 27 (50.0%) patients, respectively, were clinically free from jaundice. While the remaining 40 (68.9%) and 27 (50%) continued to have jaundice at the end of 1 and 3 months, respectively. Similarly, 35 (61.4%) patients and 34 (62.9%) patients had cholic or yellow-colored stools at the end of 1 and 3 months, respectively. At the end of 3 months, 54 patients had a mean of 5.2 ± 4.2 mg/dl, median 4.0 mg/dl of the total serum bilirubin, and it ranged from 0.4 to 16.2 mg/dl.

The TB7/TB0 ratio was calculated for all the 61 patients and the mean value was 0.73 ± 0.19, median 0.74, and ranged from a minimum of 0.25 to a maximum of 1.09. The outcome groups were divided on the basis of clinical and biochemical parameters as described above. Of the 54 patients, 27 (44.3.9%) were free of jaundice clinically at the end of 3 months, while 26 (42.6%) were clinically jaundiced at the end of 3 months. One patient had no clinical jaundice at the end of 1 month of follow-up but developed jaundice later at 3 months of follow-up. He was excluded from the analysis. Of the 54 patients, 34 (63.0%) had yellow-colored stools and 17 (31.5%) had clay-colored stools at the end of 3 months. Three patients had yellow stools at the end of 1 month, but later at 3 months, had clay-colored stools. Of the 54 patients, 23 (42.6%) had normal total serum bilirubin levels at the end of 3 months, and the remaining 31 (57.4%) had elevated serum bilirubin levels at the end of 1 month and at the end of 3-month postoperatively.

When the TB7/TB0 ratio was compared with the KP outcome, it was found that patients with a higher median value (0.856) of TB7/TB0 had significantly higher chances of persistence of clinical jaundice at the end of 1 month and at 3 months as compared to patients with a lower median value (0.615) of TB7/TB0 (P ≤ 0.0001). Similarly, it was found that patients with a higher median value (0.884) of TB7/TB0 had significantly higher chances of the persistence of clay-colored stools at the end of 1 month and 3 months as compared to patients with a lower median value (0.623) of TB7/TB0 (P = 0.0001). Patients with a higher median value (0.818) of TB7/TB0 had significantly higher chances of elevated serum bilirubin levels at the end of 1 month and at 3 months as compared to patients with a lower median value (0.575) of TB7/TB0 (P ≤ 0.0001) [Table 2].

**TB7/TB0 as a marker to predict early outcome post-Kasai portoenterostomy using receiver operating characteristics curve analysis**

The ROC curve analysis was applied to find a cutoff value of TB7/TB0 for each of the outcome. The area under the curve was highest for disappearance of clinical jaundice out of all the three outcomes which means the TB7/TB0 predicts clearance of clinical jaundice better than the color of stools and the bilirubin levels at follow up [Figure 1].

It was observed that if TB7/TB0 is >0.723, then the negative predictive value (NPV) was 84.6%. It implies that if the TB7/TB0 is <0.723, clinical jaundice will clear in 84.6% of the patients at the end of 1 and 3 months. Furthermore, if TB7/TB0 ratio >0.723, it predicts the persistence of clinical jaundice at 1 month and at 3 months with the sensitivity and specificity of 84.6% and 81.5%, respectively.

The cutoff of TB7/TB0 ratio >0.758 predicted the presence of clay-colored stools at 1 month and 3 months with the sensitivity and specificity of 82.4% and 79.4%, respectively. This implies that if the TB7/TB0 is <0.758, then the child is likely to pass cholic stools at the end of 1 and 3 months in 90% of the cases (NPV 90%).

A cutoff of TB7/TB0 ratio >0.706 predicted the presence of elevated bilirubin at 1 month and 3 months with the sensitivity and specificity of 80.7% and 78.3%, respectively. It was found that if the TB7/TB0 is <0.706, the serum bilirubin will be normal at the end of 1 and 3 months in 75% of the patients (NPV 75%) [Figure 1].

**Relation of TB7/TB0 with the histological changes**

The difference in the TB7/TB0 ratio between patients with varying severity of cholestasis (P = 0.01), hepatocellular damage (P = 0.03), portal inflammation (P = 0.04), and portal fibrosis (P = 0.02) was significant. However, the difference in TB7/TB0 ratio was not significant among patients with varying severity of

**Table 2: Relation of TB7/TB0 ratio with the outcome**

| Outcome                                      | n   | TB7/TB0                  |
|----------------------------------------------|-----|--------------------------|
| Clinical jaundice                            |     |                          |
| Absent at 1 month and absent at 3 months     | 27  | 0.6154 (0.2532-0.8451)   |
| Present at 1 month and present at 3 months  | 26  | 0.8562 (0.3818-1.0990)   |
| P                                            |     | <0.0001                  |
| Color of the stools                          |     |                          |
| Yellow at 1 month and yellow at 3 months    | 34  | 0.6227 (0.2532-0.9820)   |
| Clay at 1 month and clay at 3 months        | 17  | 0.8843 (0.5160-0.9919)   |
| P                                            |     | 0.0001                   |
| Serum bilirubin levels                       |     |                          |
| Normal at 1 month and normal at 3 months    | 23  | 0.5753 (0.2531-0.8136)   |
| Elevated at 1 month and elevated at 3 months| 31  | 0.8187 (0.3818-1.1099)   |
| P                                            |     | <0.0001                  |

Data presented as median (minimum-maximum)
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bile duct proliferation ($P = 0.07$) or with portal edema ($P = 0.08$) [Tables 3a and b].

**DISCUSSION**

BA patients if left untreated, die of hepatic decompensation, esophageal variceal bleeding, or infection within 19 months.\(^1\) Some degree of hepatic fibrosis is invariably present at initial surgery and can be graded histologically. About 25% of infants improve after KP and can live a near normal life. The remaining may progress to liver failure that can be salvaged only by LT.\(^{4,5}\)

**Predictive markers of successful Kasai operation**

The results of liver transplantation for BA were reported to be poorer in children who had previously undergone a Kasai operation.\(^{14}\) Arguments against a primary liver transplant suggested that it would deprive some children of the possibility of living with the native liver while the long-term effects of immunosuppression over decades of life are still unknown. Furthermore, a primary liver transplant would obviously dramatically increase the need for donor organs at a time when the shortage of organs is still an unresolved problem.\(^{10}\) In many countries, facilities for pediatric liver transplantation are not well developed. The ability to reliably predict outcomes would be invaluable when planning the management of BA, that is, early referral to a transplant center, optimization of medical and nutritional therapy, education and counseling of the parents, etc.

In a retrospective study by Rodeck et al., the ROC analysis determined a cutoff for bilirubin concentrations of 57 $\mu$mol/l at 6-week postoperatively for event-free survival with a sensitivity of 80% and a specificity of 78.6%. The hepatic iminodiacetic acid scan excretion values at 6-month postoperatively were also compared. The 5-year event-free survival rate was found to be 100% in the group with good tracer excretion and a bilirubin concentration of $<57$ $\mu$mol/l and 27% for the other group (log-rank test, $P < 0.0001$).\(^9\)

Another study by Baruah et al.,\(^{15}\) in 2015, supports the same conclusion that the reduction in the levels of serum

Figure 1: Receiver operating characteristics curve showing the predictive ability of TB7/TB0 ratio using clinical outcomes, namely, (a) clinical jaundice, (b) color of the stools, and (c) total serum bilirubin. Sn: Sensitivity, Sp: Specificity, CC: Classified correctly, LR: Likelihood ratio, NPV: Negative predictive value, AUC: Area under the curve, CI: Confidence interval
Table 3a: Relation of histological characteristics with TB7/TB0 (n=61)

| Histological characteristic   | Mild          | Moderate       | Severe         | P     |
|-------------------------------|---------------|----------------|----------------|-------|
| Cholestasis (n)               | 4             | 44             | 13             |       |
| TB7/TB0                       | 0.79 (0.28-0.85) | 0.71 (0.25-0.99) | 0.89 (0.51-1.10) | 0.01  |
| Hepatocellular damage (n)     | 15            | 38             | 8              |       |
| TB7/TB0                       | 0.71 (0.44-0.99) | 0.76 (0.25-1.10) | 0.89 (0.72-1)  | 0.03  |
| Bile duct proliferation (n)   | 9             | 39             | 13             |       |
| TB7/TB0                       | 0.69 (0.44-0.95) | 0.72 (0.25-1)  | 0.89 (0.28-1.10) | 0.07  |
| Bile duct inflammation (n)    | 17            | 24             | 20             |       |
| TB7/TB0                       | 0.62 (0.45-0.95) | 0.76 (0.25-1.10) | 0.83 (0.58-0.98) | 0.009 |
| Portal edema (n)              | 23            | 34             | 4              |       |
| TB7/TB0                       | 0.64 (0.25-1.10) | 0.76 (0.45-0.99) | 0.86 (0.82-0.94) | 0.08  |
| Portal inflammation (n)       | 15            | 28             | 18             |       |
| TB7/TB0                       | 0.69 (0.25-0.95) | 0.76 (0.38-1)  | 0.82 (0.56-1.10) | 0.04  |

TB7/TB0-ratio of total serum bilirubin on 7th postoperative day to the preoperative levels. Data presented as median (minimum-maximum)

Table 3b: Relation of portal fibrosis (Okhuma’s classification) to TB7/TB0 (n=61)

| Portal fibrosis                        | n   | TB7/TB0     |
|-----------------------------------------|-----|-------------|
| Portal fibrous expansion or periportal  | 15  | 0.62 (0.44-0.98) |
| fibrous septae extension present        |     |             |
| Bridging fibrosis                       | 13  | 0.74 (0.28-0.97) |
| Incomplete cirrhosis                    | 19  | 0.77 (0.25-0.99) |
| Cirrhosis                               | 14  | 0.90 (0.51-1.10) |
| P                                       |     | 0.02        |

TB7/TB0-ratio of total serum bilirubin on 7th postoperative day to the preoperative levels. Data presented as median (minimum-maximum)

Bilirubin is an obvious indicator of clinical outcome as seen in this study. With the good clinical outcome, the levels of transaminases and alkaline phosphatase also show a fall that may not be seen with poor outcome. However, no clinical cutoff value of liver function tests was suggested.

Goda et al.[7] studied 54 patients of BA and determined cutoff values of the liver function test. The most reliable cutoffs determined by ROC analysis in this study were DB of 0.7 mg/dl at 2 months (sensitivity: 93%, specificity: 75%) and aspartate transaminase (AST) of 94 IU/L at 2 months (sensitivity: 87%, specificity: 71%). The 54 cases were divided into three subgroups according to the cutoff values: Group G (good) with DB and AST < cutoffs (n = 16; Group I: II = 1:15), Group M (moderate) with DB or AST > cutoffs (n = 9; Group I: II = 4:5), and Group P (poor) with DB and AST ≥ cutoffs (n = 29; Group I: II = 25:4). The 15-year survival rate in Groups G, M, and P was 94%, 44%, and 22%, respectively (P < 0.001). A combination of serum DB and AST at 2 months after Kasai’s operation was found to be a reliable predictor of long-term BA outcome.

Yanchar et al.[8] also reported a predictive value of bilirubin 4-month postoperatively as the earliest time for predicting prognosis. The bilirubin concentrations described ranged from 14 to 46 μmol/l, and the rate of bilirubin decline was 2.6 ± 1.5–10.8 ± 3.0 μmol/l/d, respectively. Other parameters of liver function tests apart from serum bilirubin have also been studied as predictive factors.

In this study, TB7/TB0 proved to be a simple marker for predicting early the probable outcome post KP. The clearance of clinical jaundice was taken as the most important parameter out of all the three. The ROC curves comparing the ratio with the outcome parameters showed that the plot with clinical jaundice had the maximum AUC. The difference in TB7/TB0 ratio between patients with persistence of jaundice at the end of 3 months as compared to patients who were jaundice free at 3 months was significant. If the TB7/TB0 is <0.723, clinical jaundice is likely to clear at the end of 1 month in 84.6% of the patients (NPV = 84.6%). In other words, patients with TB7/TB0 ratio of more than 0.72 are 4.5 times more likely to have persistent clinical jaundice 3 months following KP (sensitivity of 84.6% and specificity of 81.5%).

It has been believed that the prognosis following KP worsens when the age of the child at surgery is higher.[16] However, other studies[15,17] have reported that age at surgery did not have any significant influence on clearance of jaundice; rather liver histology has a greater impact and liver histology did not seem to have any correlation with age. Nio et al.[19] conducted a study on more than 1000 patients and observed that there was no influence of age up to 90 days on the clearance of jaundice. Moreover, a considerable percentage of patients who underwent late portoenterostomy (after 90 days of life) survived with their native livers for 5 and 10 years. Davenport et al.[19] also showed that age of surgery had no effect on isolated BA as compared to those with associated cysts or splenic malformation.

In the study being reported, it was found that cholestasis was present in all patients and hepatocellular damage of moderate degree was present in majority (62.3%)
of the patients. Similarly, 63.9% and 55.7% patients had moderate degree of bile duct proliferation and portal edema, respectively. All patients had bile duct inflammation with almost equal distribution of patients in the mild, moderate, and severe groups. Portal inflammation was moderate to severe in more than half of the patients. When the TB7/TB0 ratio was compared with the severity of histopathological changes, it was found that the difference in TB7/TB0 ratio between patients with varying severity of cholestasis, hepatocellular damage, portal inflammation, and portal fibrosis was significant. However, the difference in TB7/TB0 ratio was not significant among patients with varying severity of bile duct proliferation and portal edema.

**Conclusions**

The rapidity of fall in the total serum bilirubin level within 1 week was able to predict the outcome post-KP. This TB7/TB0 ratio is easy to calculate and can be used objectively to explain to the parents the expected result of Kasai operation within 7 days of surgery or at the time of discharge from the hospital. This would further ensure early enrollment of the expected poor outcome cases in the liver transplant program. Hence, the ratio of total serum bilirubin on the 7th postoperative day to its preoperative levels is a reliable marker to predict early, the outcome following surgery in BA patients. The long-term outcomes including overall survival of these children with their native liver, survival at 2- and 5-year follow-up, bilirubin at 2- and 5-year postoperatively are still being studied.

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**Conflicts of interest**

There are no conflicts of interest.

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