Original Article

Impact of Cytomegalovirus Infection on Short-Term Clinical Outcomes and Operative Histopathology in Infants with Biliary Atresia: A Single-Center Prospective Cohort Study

Gayatri Munghate, Sachit Anand, Veereshwar Bhatnagar, Sandeep Agarwala, Siddhartha Datta Gupta

Background: There is limited information on the impact of cytomegalovirus (CMV) infection on clinical outcomes and operative histopathology in children with biliary atresia (BA). We hypothesized that CMV infection is associated with greater histopathological damage and unfavorable short-term clinical outcomes.

Materials and Methods: A prospective single-center study was conducted with effect from January 2011–July 2012 including all infants with BA who underwent surgery. Diagnosis of CMV infection was confirmed by serum immunoglobulin M (IgM) positivity or the presence of CMV-deoxyribonucleic acid (DNA) in the liver tissue. Four short-term outcome variables were observed. The cohort was divided into subgroups on the basis of seropositivity (IgM + or IgM−); the presence of CMV-DNA in the liver (polymerase chain reaction [PCR] + or PCR−); and composite CMV groups (Group 1 – IgM+, PCR+; Group 2 – IgM+, PCR−; Group 3 – IgM−, PCR+; and Group 4 – IgM−, PCR−). Outcomes and histopathology were compared in these subgroups.

Results: A total of 32 infants with BA were operated at a mean age of 3.5 (range: 1–6) months. Serum IgM+ and PCR+ were observed in 50% and 37.5% of the patients. Unfavorable outcomes showed a significant association with IgM+ and not PCR+. Similarly, outcomes were poor for CMV Groups 1 and 2 at 1-month follow-up. Infants with IgM+ and PCR+ showed a greater degree of histopathological damage in terms of bile duct proliferation and severe bile duct fibrosis, respectively.

Conclusion: In the present study, there was a high incidence of serum IgM+ (50%) and PCR+ of biopsy specimens (37.5%) in infants with BA. This CMV-infected subgroup was associated with greater histopathological damage and unfavorable short-term outcomes after surgery.

Keywords: Biliary atresia, cytomegalovirus, histopathology, unfavorable outcomes

Submit: 25-Jun-2021.
Revised: 26-Mar-2022.
Accepted: 06-May-2022.
Published: 26-Jul-2022.

INTRODUCTION

Biliary atresia (BA) is a progressive sclerosing cholangiopathy of newborns involving both extrahepatic and intrahepatic bile ducts.[1] The exact etiology of BA has remained elusive so far. However, several viral infections have been implicated in its etiopathogenesis. The most extensively studied viral agents are cytomegalovirus (CMV) and reovirus.[2,3]
CMV is a double-stranded deoxyribonucleic acid (DNA) virus having the propensity of causing perinatal infection, with 1%–2% of the infants infected at birth.[2] Various studies have shown a higher prevalence of CMV antibodies in the mothers of BA infants, and the presence of CMV DNA in liver tissue of these infants.[2,3] Whether it acts by direct injury to the biliary epithelium or immune-mediated damage is not known.[1,4] The aim of this study was to investigate the impact of CMV infection, diagnosed by the presence of serum CMV-Immunoglobulin M (IgM) or the presence of CMV-DNA in the liver tissue, on short-term clinical outcomes of BA. We also intend to establish a correlation between CMV infection and the degree of histopathological damage in BA.

**Materials and Methods**

This prospective study was conducted in the Department of Pediatric Surgery at our center w.e.f. January 2011–July 2012. Ethical approval was obtained from the Institutional Review Board prior to starting the study. All infants who were diagnosed with infantile obstructive jaundice and admitted for per-operative cholangiogram (POC) were selected, while only those with POC findings consistent with BA were included in the study. Children with a patent biliary tree on POC were excluded. Those who were discharged against medical advice before undergoing surgery were also excluded.

Baseline data on age at surgery, gender, demographic details, clinical features, and investigation details (including liver function tests [LFTs]) were collected. As a departmental protocol, the toxoplasma, rubella, CMV, and herpes simplex virus (TORCH) panel is routinely performed in all children with neonatal cholestasis. Detection of CMV-specific IgM in serum using enzyme-linked immune sorbent assay kit (Diamedix, Inc.® USA) forms a part of this panel and indicates active CMV infection.

**Screening for Cytomegalovirus in the Biopsy Sample**

In all participants, a wedge biopsy from the liver and biopsy of the portal plate tissue were taken. A portion of this biopsy sample was stored at −70°C and was subsequently used for detection of the CMV-deoxyribonucleic acid (DNA). DNA extraction involved digestion of the biopsy samples (utilizing a digestion buffer), DNA precipitation (using absolute ethanol containing ammonium acetate), and centrifugation. Subsequently, the supernatant was removed and the DNA pellet was dried at room temperature. DNA amplification: The extracted DNA was subjected to an initial external glycoprotein B (gB) gene polymerase chain reaction (PCR) (preincubation at 94°C for 4 min, then 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min). The following primers, taken from the envelope gB region of CMV, were used to detect a 501-bp region:

- gB 1138-5’TGACAGTGAACATGTCCGA 3’
- gB 1638-5’TGCACGAGCTTGCCAG 3’.

Subsequent to the initial gB gene PCR, nested gB gene PCR was performed. The template for nested PCR was the external PCR product. The PCR detected a ~295-bp amplicon (actually 293–297 bp, depending on the gB type of the CMV strain). The primers were as follows:

- gB 1319-5’TGGACCTGGAAACGTTTGGC 3’
- gB 1604-5’GAAACGGCGCGGGCAATCGG 3’.

Finally, the nested amplicon was subjected to gel electrophoresis and visualization in a gel documentation system.

**Histopathological Grading**

The liver biopsy slides (hematoxylin and eosin stained) of all the patients were reviewed by an expert pathologist (Sustainable Development Goal). The parameters studied were cholestasis, hepatocellular damage, bile duct proliferation, bile duct fibrosis, bile duct inflammation, portal edema, portal inflammation, and degree of portal fibrosis.[5] For all the histopathological parameters except fibrosis, the grading was done using a semi-quantitative scoring system, i.e., mild (1+), moderate (2+), and severe (3+). Fibrosis Grades 1, 2, and 3 correspond to mild portal fibrosis, porto-septal (nonbridging) fibrosis, and bridging fibrosis, respectively.[5]

**Postoperative Treatment, Outcomes, and Analysis**

In the postoperative period, all infants received oral ursodeoxycholic acid (15 mg/kg/day), phenobarbital (5 mg/kg/day), and betamethasone (10 drops/day) for 3 months. Antibiotic prophylaxis for cholangitis was also administered postoperatively for at least 6 weeks.

Clinical examination and LFTs were performed at the 1-month follow-up visit. Four outcome variables were studied: the presence of cholic stools at discharge, the presence of cholic stools at 1 month after surgery, the persistence of jaundice at 1 month after surgery, and median serum bilirubin (mg/dL) at 1 month after surgery. Children were divided into three age groups on the basis of age at surgery: groups A, B, and C consisting of children aged <60, 60–90, and >90 days. On the basis of results of the CMV serology, the
children were categorized as seropositive (IgM+) or seronegative (IgM−). Similarly, infants in whom CMV-DNA could be detected in liver and/or portal plate tissue were grouped as PCR positive (PCR+), and the rest were labeled as PCR−. The participants were further subgrouped (composite CMV groups) as follows: group 1 – IgM+, PCR+; Group 2 – IgM+, PCR−; Group 3 – IgM−, PCR+; and Group 4 – IgM−, PCR−. All the outcome variables were compared among the age groups, serology groups, PCR groups, and composite CMV groups (both serology and PCR). In addition, the histopathological grades (for each histopathological feature) were correlated with age group, serology group, and PCR group.

Data were expressed as numbers, proportion, and averages (mean or median). The qualitative variables were analyzed using Fisher’s exact test. Differences in the median values of two groups of patients were analyzed using Wilcoxon rank-sum test. P < 0.05 was considered to be statistically significant. Data entry was done using Microsoft Excel and analysis was performed using Stata/SE 12.0.

**Results**

Thirty-six infants were admitted during the study period as per the inclusion criteria. Of these, two were discharged against medical advice before undergoing surgery and two children had a patent biliary tree on cholangiogram. Thus, 32 (M:F = 26:6) had BA and underwent Kasai’s portoenterostomy. The mean age at surgery was 3.5 (range: 1–6) months. The age Groups A, B, and C consisted of 12.5% (4/32), 21.9% (7/32), and 65.6% (21/32) of the patient population, respectively. TORCH assay showed 50% (16/32) of the patients to be IgM+. On PCR, CMV-DNA was detected in 37.5% (12/32) of the patients. The composite CMV Groups 1, 2, 3, and 4 consisted of 5, 11, 7, and 9 children, respectively [Figure 1]. Overall, 14/16 (87.5%) of the IgM + and 11/12 (91.7%) of the PCR + infants belonged to the age group C. Baseline LFTs showed no significant difference among the various age groups and serology groups [Table 1]. Apart from significantly raised levels of alanine transaminase (ALT), none of the other biochemical parameters showed an association with the presence of CMV-DNA [Table 1].

The four outcome variables were studied in all children. Higher values of median serum bilirubin at 1 month after surgery showed a significant association (0.044) with advanced age at surgery [Table 2]. None of the other outcome variables showed a significant association with age groups.

**Relationship of cytomegalovirus-serology and cytomegalovirus-deoxyribonucleic acid with outcomes**

Children with IgM+ had a significant association with unfavorable clinical outcomes [Table 3]. On the other hand, those with PCR + showed no association with unfavorable clinical outcomes. Upon combined analysis (composite CMV groups), no significant difference among the four subgroups was observed in terms of the passage of acholic stools at discharge [Table 3]. However, unfavorable outcomes at 1-month follow-up including the presence of acholic stools, the persistence of jaundice, and higher median serum bilirubin levels were significantly associated with CMV groups having positive serology (Groups 1 and 2). On further subgroup analysis, clinical outcomes were significantly different among Groups 1 versus 3, 1 versus 4, 2 versus 3, and 2 versus 4. The difference in the outcomes was not statistically significant among Groups 1 versus 2 and 3 versus 4. There were two early postoperative deaths in the cohort. IgM+ (1/2) or PCR+ (1/2) showed no association with mortality.

**Histopathology**

The distribution of patients according to the histopathological grading is shown in Figure 2. The severity of histopathological damage was correlated with advanced age at surgery (age >90 days), presence of IgM in serum, and presence of CMV-DNA in the liver [Table 4]. Advanced age showed a significant correlation with increased severity of hepatocellular damage, bile duct inflammation, and portal inflammation. IgM+ and PCR+ showed a significant association with bile duct proliferation and severe bile duct fibrosis, respectively. Similar to IgM+, the CMV Groups 1 and 2 also showed a significant association with bile duct proliferation. None of the patients had the presence of CMV-inclusion bodies.
Following the initial hypothesis by Landing,[6] various studies have demonstrated the possible role of hepatotropic viruses in the etiopathogenesis of BA.[3,7-9] Of all the hepatotropic viruses, the role of CMV has been extensively studied in multiple clinical and experimental studies and has shown contrasting results.[1,3,10,11] Zani et al. have highlighted CMV-associated BA to be a distinct subgroup with specific clinico-histopathological characteristics and poor response after portoenterostomy.[1] On the other hand, Rauschenfels et al. have shown the presence of all hepatotropic viruses (including CMV) to be a secondary phenomenon rather than having a major role in the etiopathogenesis of BA.[11]

The incidence of IgM+ among the BA infants in the present series was 50% (16/22). Similar results of high IgM+ have been observed in previous studies from different parts of the world.[2,3,12-14] Goel et al.[15] from India have also demonstrated a high incidence (47%) of IgM+ in infants with BA. In contrast, few have also depicted a lower incidence of IgM+ among these children. [1] Apart from major geographical differences, these variations in seropositivity reflect the high incidence of false-positive and false-negative results. The false positivity can occur as a result of rheumatoid factor (IgM of the infant against maternal IgG) and cross-reaction to other viruses from the Herpes family. False-negative results may be due to high levels of maternal IgG competing with low levels of fetal IgM. [2] Therefore, as in the present study, it is always advisable to use more than one method for the diagnosis of CMV infection in newborns. [2]

In the present study, CMV-DNA was isolated in the liver and/or portal plate specimens of 37.5% (12/32) of the patients. Similar results have been demonstrated

**Table 1: Biochemical parameters in different patient groups based on age at surgery, serology, and polymerase chain reaction results**

| Groups | Serum total bilirubin; mg % | Serum albumin; g % | AST; IU/L | ALT; IU/L | ALP; IU |
|--------|----------------------------|-------------------|----------|----------|---------|
| <60 days (A) | 8.95 (6.3–12.1) | 4.15 (3.2–4.8) | 277.5 (201–516) | 157.5 (67–500) | 2079 (1294–2187) |
| 60–90 days (B) | 10.4 (8.7–15.1) | 4.3 (3.6–4.9) | 306 (188–434) | 177 (111–358) | 1283 (898–1690) |
| >90 days (C) | 12.3 (4–22.4) | 3.6 (2.3–4.7) | 301 (70–766) | 192 (67–630) | 967 (260–33580) |
| P | 0.2698 | 0.589 | 0.9626 | 0.9093 | 0.590 |

**Parameters in different age groups**

| Positive (n=16; 50%) | 10.35 (4–22.4) | 3.6 (2.3–4.6) | 269 (98–766) | 137 (67–529) | 1068.5 (519–2575) |
| Negative (n=16; 50%) | 11.45 (6.3–22.4) | 4 (3.2–4.9) | 312 (70–654) | 203 (67–630) | 1288.5 (264–3580) |
| P | 0.5215 | 0.0853 | 0.6510 | 0.1415 | 0.8505 |

**Parameters in different serology groups**

| Positive (n=12; 37.5%) | 11.25 (4–20.9) | 3.9 (3–4.9) | 359 (188–554) | 301.5 (130–630) | 1229 (260–1773) |
| Negative (n=22; 68.75%) | 10.79 (5.8–22.4) | 3.7 (2.3–4.8) | 260.5 (170–766) | 135 (67–529) | 1209 (468–3580) |
| P | 0.7702 | 0.4347 | 0.0565 | 0.0031* | 0.4137 |

*Significant difference. AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, PCR: Polymerase chain reaction

**Table 2: Comparison of clinical outcomes among different age groups**

| Age group | Cholic stools at discharge, n (%) | Cholic stools at one month, n (%) | Persistent Jaundice at 1 month, n (%) | Median (range) serum bilirubin at one month; mg % |
|-----------|----------------------------------|----------------------------------|--------------------------------------|-----------------------------------------------|
| <60 days (n=4) | 3 (75) | 2 (50) | 2 (50) | 0.9 (0.8–14.5) |
| 60–90 days (n=7) | 6 (85.71) | 6 (85.71) | 1 (14.29) | 4.35 (0.7–8.7) |
| >90 days (n=21) | 12 (57.14) | 10 (52.63) | 12 (63.16) | 5.9 (0.9–19.4) |
| P | 0.473 | 0.359 | 0.086 | 0.044* |

*Significant difference

**Figure 2:** Distribution of patients according to pathological grading. Grades 1, 2 and 3 correlate with the increasing severity of histopathological damage

**Discussion**

Following the initial hypothesis by Landing,[6] various studies have demonstrated the possible role of hepatotropic viruses in the etiopathogenesis of BA.[3,7-9] Of all the hepatotropic viruses, the role of CMV has been extensively studied in multiple clinical and experimental studies and has shown contrasting results.[1,3,10,11] Zani et al. have highlighted CMV-associated BA to be a distinct subgroup with specific clinico-histopathological characteristics and poor response after portoenterostomy.[1] On the other hand, Rauschenfels et al. have shown the presence of all hepatotropic viruses (including CMV) to be a secondary phenomenon rather than having a major role in the etiopathogenesis of BA.[11] The incidence of IgM+ among the BA infants in the present series was 50% (16/22). Similar results of high IgM+ have been observed in previous studies from different parts of the world.[2,3,12-14] Goel et al.[15] from India have also demonstrated a high incidence (47%) of IgM+ in infants with BA. In contrast, few have also depicted a lower incidence of IgM+ among these children.[1] Apart from major geographical differences, these variations in seropositivity reflect the high incidence of false-positive and false-negative results. The false positivity can occur as a result of rheumatoid factor (IgM of the infant against maternal IgG) and cross-reaction to other viruses from the Herpes family. False-negative results may be due to high levels of maternal IgG competing with low levels of fetal IgM.[2] Therefore, as in the present study, it is always advisable to use more than one method for the diagnosis of CMV infection in newborns.[2] In the present study, CMV-DNA was isolated in the liver and/or portal plate specimens of 37.5% (12/32) of the patients. Similar results have been demonstrated
by De Tommaso et al. [2] However, the study by Jevon and Dimmick [16] found no association of CMV-DNA in children with BA. These findings can be explained by an overall low incidence of CMV infection among the Canadian population. In addition, the use of formalin-fixed tissue rather than fresh biopsy samples in their study might have decreased the detection rate of the CMV-DNA. Another study demonstrated the PCR positivity in the liver tissues of 23/50 patients with neonatal hepatitis and only 2/26 patients with BA [17]. The exact reason behind this low detection rate among the BA infants is not known, as PCR+ rate of as high as 50% has been observed by a study in the Indian population [15]. Nevertheless, the higher accuracy of CMV-DNA detection in the liver over CMV-IgM+ in serum has been well established in the literature [2,15]. In our study, 7/32 (22%) of the patients had PCR+ but were IgM−. Similarly, there were 11/32 (34%) children with PCR− and IgM+. This discordance again re-affirms the importance of using more than one test for diagnosis.

The majority of the IgM+ and PCR+ infants were operated at an advanced age (>90 days) in our cohort. Zani et al. [1] have also reported similar results, where the CMV-infected infants were almost 2 weeks older than the noninfected ones at the time of portoenterostomy. The possible reasons for this can be a delayed referral owing to misinterpretation of CMV infection as the cause of hepatitis or CMV infection itself causing a late-onset BA [3]. However, these possibilities might need further testing, as there was an overall majority (65.6%) in terms of advanced age at surgery in our study. Apart

### Table 3: Correlation of clinical outcomes with different variables including seropositivity, polymerase chain reaction positivity, and composite cytomegalovirus groups

| Variables | n | Cholic stools at discharge, n (%) | Cholic stools at 1 month, n (%) | Persistent jaundice at 1 month, n (%) | Serum bilirubin at 1 month, median (range) |
|-----------|---|----------------------------------|---------------------------------|--------------------------------------|------------------------------------------|
| Positive  | 16 | 7 (33.33)                        | 5 (27.78)                       | 12 (80)                              | 9.8 (1.1–19.4)                           |
| Negative  | 16 | 14 (66.67)                       | 13 (72.22)                      | 3 (20)                               | 1.1 (0.7–10)                            |
| P         |    | 0.023*                           | 0.008*                          | 0.000*                               | 0.0003*                                 |

Correlation of outcomes with results of PCR

| Positive | 12 | 8 (38.10)                        | 7 (38.89)                       | 5 (33.33)                            | 2 (0.7–18.9)                           |
| Negative | 20 | 13 (61.90)                       | 11 (61.11)                      | 10 (66.67)                           | 4.5 (0.8–19.4)                        |
| P        |    | 1.000                            | 1.000                           | 0.610                                | 1.000                                  |

Correlation of outcomes with CMV groups

| 1Serology+/PCR+ | 5  | 2 (9.52)                        | 1 (5.56)                       | 5 (33.33)                            | 12.2 (5.1–18.9)                          |
| 2Serology+/PCR− | 11 | 5 (23.81)                       | 4 (22.22)                      | 7 (46.67)                            | 9.05 (1.1–19.4)                          |
| 3Serology−/PCR+ | 7  | 6 (28.57)                       | 6 (33.33)                      | 0 (0.00)                             | 1.2 (0.7–2)                             |
| 4Serology−/PCR− | 9  | 8 (38.10)                       | 7 (38.89)                      | 3 (20)                               | 1.1 (0.8–10)                            |
| P         |    | 0.081                            | 0.016*                         | 0.000*                               | 0.0031*                                 |

P between 1 and 3 and 1 and 4

P between 2 and 3 and 2 and 4

P between 1 and 2 and 3 and 4

*Significant difference. PCR: Polymerase chain reaction, CMV: Cytomegalovirus, 1,2,3 and 4: Four composite CMV groups

### Table 4: Histopathological grading and correlation with age groups, seropositivity for cytomegalovirus, and polymerase chain reaction positivity

| Histopathological feature | Advanced age (>90 days); P | Seropositivity; P | PCR positivity; P |
|--------------------------|----------------------------|------------------|-------------------|
| Cholestasis              | 1.000 (0.43)               | 0.690 (0.008)    | 0.821 (0.752)     |
| Hepatocellular damage    | 0.0184* (0.643)            | 0.690 (0.008)    | 0.821 (0.752)     |
| Bile duct proliferation  | 0.498* (0.722)             | 0.498* (0.722)   | 0.498* (0.722)    |
| Bile duct fibrosis       | 1.000 (1.000)              | 1.000 (1.000)    | 1.000 (1.000)     |
| Bile duct inflammation   | 0.032* (0.220)             | 0.032* (0.220)   | 0.032* (0.220)    |
| Portal edema             | 0.276 (0.346)              | 0.276 (0.346)    | 0.276 (0.346)     |

*Significant difference. PCR: Polymerase chain reaction

Our study, 7/32 (22%) of the patients had PCR+ but were IgM−. Similarly, there were 11/32 (34%) children with PCR− and IgM+. This discordance again re-affirms the importance of using more than one test for diagnosis. The majority of the IgM+ and PCR+ infants were operated at an advanced age (>90 days) in our cohort. Zani et al. [1] have also reported similar results, where the CMV-infected infants were almost 2 weeks older than the noninfected ones at the time of portoenterostomy. The possible reasons for this can be a delayed referral owing to misinterpretation of CMV infection as the cause of hepatitis or CMV infection itself causing a late-onset BA [3]. However, these possibilities might need further testing, as there was an overall majority (65.6%) in terms of advanced age at surgery in our study. Apart
from higher levels of ALT in PCR+ patients, none of the other LFT parameters showed a significant association with CMV infection. These results are similar as depicted by Goel et al.\[15\] and highlight the possibility of severe degree of fibrosis in these infants. In fact, the presence of severe bile duct fibrosis in PCR+ patients in our cohort confirms the observations by previous studies.\[1]\n
Of the four outcome variables, only higher serum bilirubin levels were found to have an association with advanced age in our study. This limited correlation with clinical outcomes differs from that of previous studies. It is possible that small sample size and disproportionately high number of patients with advanced age might be responsible for these results. It must also be noted that in spite of the above factors, 53% (17/32) of the patients could achieve biliary drainage at 1 month after surgery. Upon correlating the CMV infection status with outcomes, BA infants with IgM+ status showed a significant association with unfavorable clinical outcomes. No significant association was observed between the PCR+ status and outcomes. Furthermore, the subgroup analysis among the composite CMV groups revealed that the outcomes at 1-month follow-up largely depend upon the serology status. Zani et al.\[1\] also observed similar findings of significantly lower rates of jaundice clearance among the IgM+ infants. Native liver survival and true survival rates were also significantly low in this subgroup. Similarly, another study by Shen et al.\[14\] had demonstrated lower rates of disappearance of jaundice and higher incidence of reflux cholangitis among the CMV-infected group. One might argue that the presence of CMV-DNA in the liver gives a more accurate representation of active infection in BA as compared to IgM positivity; still, the outcomes showed a correlation with the latter and not the former. The possible explanation for this finding is the ability of this virus to lodge in the tissue and establish a latent infection by evading the immune system. Delgado et al.\[18\] have documented a similar finding in liver transplant patients where a significant number of PCR+ patients never developed symptomatic infection. Therefore, merely viral DNA isolation from the liver cannot be taken as an indicator of active infection, and alike Shen et al.,\[14\] further studies must also focus on the markers of viral replication (e.g., CMV pp65).

Upon correlating the severity of histopathological changes with CMV infection, severe degrees of bile duct proliferation and bile duct fibrosis were significantly associated with IgM+ and PCR+, respectively. Shen et al.\[14\] and Zani et al.\[1\] have demonstrated similar results of pronounced inflammatory cell infiltration, greater degrees of hyperplasia of bile canaliculi, and increased liver fibrosis in patients with CMV infection. These findings depict a greater extent of pathological damage in CMV-infected infants.\[1]\n
The results of the present study should be interpreted within the context of few limitations. First, due to the rarity of the disease, the sample size of our cohort was small. Subgrouping them on the basis of their serology status and presence of CMV-DNA further limits the number of patients and prevents us from reaching any definite conclusions. Children with neonatal hepatitis and healthy controls were also not recruited in the present study. Detection of IgM and isolation of DNA in these children will provide further insight regarding the role of CMV in BA and non-BA diseases (neonatal hepatitis) in the future. Second, the timing of infection (prenatal or perinatal) could not be determined as data regarding maternal screening for CMV infection during pregnancy were unavailable. Furthermore, due to the nature of the study, IgM detection and DNA isolation were performed at the time of admission and not within the first 2 weeks of life. The establishment of the timing of the infection in further studies will also provide a better understanding of the role of congenital CMV infection in the pathogenesis of BA. Third, a qualitative PCR technique was used in the present study to isolate CMV-DNA in the liver. It is not sure whether the source of the virus was hepatocytes, bile duct cells, or circulating leukocytes. Quantitative PCR and in situ hybridization techniques will provide an answer to this in the future. Furthermore, the markers of viral replication (CMV pp65) need to be studied along with other diagnostic tests to improve the discordance between IgM detection and CMV-DNA isolation. Fourth, the role of adjuvant antiviral therapy also needs to be confirmed in future studies as it may provide definite advantage in terms of native liver survival or overall survival. Finally, the results of this study are promising and provide information about the impact of CMV infection (in terms of IgM+ and PCR+) on short-term clinical outcomes and operative histopathology. However, the long-term outcomes in these patients need to be studied to know the further course of the CMV infection.

**Conclusion**

In the present study, there is a high incidence of serum IgM+ (50%) and PCR+ of biopsy specimens (37.5%) in infants with BA. Although a poor correlation is observed between serum IgM and CMV-DNA in the liver, this virus-infected subset of BA is associated with advanced age (>90 days) and significantly elevated ALT levels at presentation. In terms of histopathological damage, the
severity of bile duct proliferation and bile duct fibrosis is more in infants with IgM+ and PCR+, respectively. However, only IgM+ and not the PCR+ significantly correlates with unfavorable early postoperative clinical outcomes of BA.

**Financial support and sponsorship**
Nil.

**Conflicts of interest**
There are no conflicts of interest.

**REFERENCES**

1. Zani A, Quaglia A, Hadzić N, Zuckerman M, Davenport M. Cytomegalovirus-associated biliary atresia: An aetiological and prognostic subgroup. J Pediatr Surg 2015;50:1739-45.

2. De Tommaso AM, Andrade PD, Costa SC, Escanhoela CA, Hessel G. High frequency of human cytomegalovirus DNA in the liver of infants with extrahepatic neonatal cholestasis. BMC Infect Dis 2005;5:108.

3. Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A. The viral association of neonatal cholestasis in Sweden: A possible link between cytomegalovirus infection and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 1998;27:57-64.

4. Kosai K, Kage M, Kojiro M. Clinicopathological study of liver involvement in cytomegalovirus infection in infant autopsy cases. J Gastroenterol Hepatol 1991;6:603-8.

5. Gupta L, Gupta SD, Bhatnagar V. Extrahepatic biliary atresia: Correlation of histopathology and liver function tests with surgical outcomes. J Indian Assoc Pediatr Surg 2012;17:147-52.

6. Landing BH. Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst-the concept of infantile obstructive cholangiopathy. Prog Pediatr Surg 1974;6:113-39.

7. Bangaru B, Morecki R, Glaser JH, Gartner LM, Horwitz MS. Comparative studies of biliary atresia in the human newborn and reovirus-induced cholangitis in weaning mice. Lab Invest 1980;43:456-62.

8. Drut R, Gómez MA, Drut RM, Cueto RE, Lojo M. Human papillomavirus, neonatal giant cell hepatitis and biliary duct atresia. Acta Gastroenterol Latinoam 1998;28:27-31.

9. Riepenhoff-Talty M, Schaeckel K, Clark HF, Mueller W, Uhnoo I, Rossi T, et al. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res 1993;33:394-9.

10. Wang W, Zheng S, Shong Z, Zhao R. Development of a guinea pig model of perinatal cytomegalovirus-induced hepatobiliary injury. Fetal Pediatr Pathol 2011;30:301-11.

11. Rauschenfels S, Krassmann M, Al-Masri AN, Verhagen W, Leonhardt J, Kuebler JF, et al. Incidence of hepatotropic viruses in biliary atresia. Eur J Pediatr 2009;168:469-76.

12. Soomro GB, Abbas Z, Hassan M, Luck N, Memon Y, Khan AW. Is there any association of extra hepatic biliary atresia with cytomegalovirus or other infections? J Pak Med Assoc 2011;61:281-3.

13. Moore SW, Zabiegaj-Zwick C, Nel E. Problems related to CMV infection and biliary atresia. S Afr Med J 2012;102:890-2.

14. Shen C, Zheng S, Wang W, Xiao XM. Relationship between prognosis of biliary atresia and infection of cytomegalovirus. World J Pediatr 2008;4:123-6.

15. Goel A, Chaudhari S, Sutar J, Bhonde G, Bhatnagar S, Patel V, et al. Detection of cytomegalovirus in liver tissue by polymerase chain reaction in infants with neonatal cholestasis. Pediatr Infect Dis J 2018;37:632-6.

16. Jevon GP, Dimmick JE. Biliary atresia and cytomegalovirus infection: A DNA study. Pediatr Dev Pathol 1999;2:11-4.

17. Chang MH, Huang HH, Huang ES, Kao CL, Hsu HY, Lee CY. Polymerase chain reaction to detect human cytomegalovirus in livers of infants with neonatal hepatitis. Gastroenterology 1992;103:1022-5.

18. Delgado R, Lumbrales C, Alba C, Pedraza MA, Otero JR, Gómez R, et al. Low predictive value of polymerase chain reaction for diagnosis of cytomegalovirus disease in liver transplant recipients. J Clin Microbiol 1992;30:1876-8.