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Original Article

Clinicobiochemical Parameters of Cytomegalovirus IgM and IgG positive Biliary Atresia and their Relation with Serological Titer in Infants

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Abstract

Cytomegalovirus (CMV) associated Biliary atresia (BA) is one of the clinical classification of Biliary atresia (BA). There is a hypothesis that CMV IgM positive BA is a clinically different entity and prognosis is poor. The aim of this study was to evaluate the clinical and biochemical parameters of CMV IgM and CMV IgG positive BA in one to six month old infants and their relation with serological titer. This cross-sectional study was carried out from January 2019 to June 2022 in the department of Pediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. A total of 48 infants were included in this study as study subject who were diagnosed as biliary atresia with positive CMV IgM & CMV IgG. After taking written informed consents data were taken from parents or legal guardians by using a structured questionnaire. Data were analyzed by statistical package for social sciences (SPSS), version- 23. Majority of the cases (58.3%) were in 2-4 month of age group, 70.8% were male and male-female ratio was almost 2.5:1. Regarding birth history most of the infants (95.8%) were term baby and two-third (66.7%) of them was appropriate for gestational age (AGA). This study observed the onset of jaundice among the infants, here 60.4% of them detected jaundice jaundice within 7 days, 22.9% within 7-14 days and 16.7% after 14 days. About three fourth (73%) of infants presented with intermittent pale stool and more than one-fourth (27%) had persistent pales tool. One fourth 12(25%) patient had features of coagulopathy. The mean of total bilirubin, direct bilirubin, alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT) were 11.89±4.0, 8.27±3.42, 162.67±103.09 and 669.46±543.57 respectively; The mean of the titers of CMV IgM and CMV IgG were 50.84±33.09 and 82.40±53.79 respectively. The prolong international normalised ratio (INR) was in 30.8% of infants and mean INR was 2.44±2.54. Study finds that CMV IgM titer level was correlated with total bilirubin (r=-0.256; p<.05) and direct bilirubin (r=-0.365; p<.05); where CMV IgG titer level was correlated with age (r=-0.362; p<.05) and INR (r=0.271; p<.05). It may be concluded as increase in age would increase titer of IgG and increase in titer of IgG is associated with increase coagulopathy. Elevated levels of CMV IgM titer correlated with bilirubin level or cholestasis.

Keywords: *Biliary atresia, cytomegalovirus IgM, cytomegalovirus IgG, clinicobiochemical parameters, antibody titer.*

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INTRODUCTION

Biliary atresia (BA) is a destructive, obliterative cholangiopathy of the newborn characterized by a variable degree of obliteration of both intrahepatic and extrahepatic bile ducts eventually causing severe cholestasis progressing to biliary cirrhosis.[1]

Japanese Association of Pediatric Surgeons classified biliary atresia (BA) into three types based on the extrahepatic bile duct obstruction. Clinically, biliary atresia (BA) is classified as isolated, typical biliary atresia (80%), cystic biliary atresia (5-10%), Biliary atresia-splenic malformation syndrome (BASM: 5-15%), cytomegalovirus (CMV) associated biliary atresia (5-10%).[2] Clinical course & prognosis of these four type of biliary atresia (BA) may vary whether these different type have different causes is

unknown.[2] In this study our concern is about cytomegalovirus (CMV) associated biliary atresia (BA) on the basis of cytomegalovirus IgM, cytomegalovirus IgG positivity . It is believed that biliary atresia (BA) is disorder of multifactorial etiology. Infectious etiology, especially viral infection proposed as precipitating factor of biliary atresia (BA) by several studies. Infection targeting the bile duct induced an auto-immune response leads to chronic fibrosclerosing injury.[3,4] Among several viruses cytomegalovirus (CMV) considered to be the most common virus related to biliary atresia (BA).[5, 6] At first serological evidence of cytomegalovirus (CMV) exposure support this speculation, later polymerase chain reaction (PCR) methods used for the detection of cytomegalovirus (CMV).[7] However, direct virus isolation or inclusion bodies could not be possible yet to conclude the hypothesis.[8] 30–40% of infants with biliary atresia (BA) showed Serological evidence of cytomegalovirus (CMV) infection in study from different countries.[9,10,11] Cytomegalovirus (CMV) -specific liver Tell response was also found in more than 50 % infant with biliary atresia (BA) with positive correlation of this laboratory finding with plasma cytomegalovirus IgM levels.[12] Recently a meta-analysis showed higher prevalence (25.4%) of cytomegalovirus (CMV) infection in infants with biliary atresia (BA). Moreover, the prevalence rate of cytomegalovirus (CMV) infection is higher in Asia than in Europe & this high prevalence have relation with lower socioeconomic status.[13] Zani A et al conducted a study in 2015, showed cytomegalovirus (CMV) associated biliary atresia (BA) infants clinically different from non cytomegalovirus (CMV) associated biliary atresia (BA), relatively older at Kasai portoenterostomy (KPE), more severe biochemical derangement in cytomegalovirus (CMV) associated biliary atresia (BA) infants.[14] Also recently a trial antiviral therapy showed better outcome in cytomegalovirus (CMV) associated biliary atresia (BA) . The aim of this study was to evaluate clinical, biochemical parameters and serological titers of infants with biliary atresia (BA) & to determine correlation between cytomegalovirus IgM, cytomegalovirus IgG titer level and different clinical & biochemical parameters in cytomegalovirus (CMV) associated biliary atresia(BA) whether any influence on severity & outcome of the disease.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Paediatric Gastroenterology and Nutrition, BSMMU during the period from January 2019 to June 2022. A total of 48 infants were included in this study as study population. Considering selection criteria infants of diagnosed biliary atresia with cytomegalovirus IgM and cytomegalovirus IgG positive were selected from the the Department of Paediatric Gastroenterology and Nutrition study subjects. Parents or legal guardians of infants were considered as respondents. A structured questionnaire and a data sheet were designed with a view to collect data from the respondents. Then detail clinical history, physical examination findings and investigation reports were recorded in the preformed data sheet. With all aseptic precautions venous blood was drawn for laboratory work up. Cytomegalovirus IgM and cytomegalovirus IgG antibodies were measured using Chemiluminescence ELISA technique at Virology Department of BSMMU. A cytomegalovirus IgM positive was defined as anti-CMV IgM > = 22AU/ml and a positive cytomegalovirus IgG was defined as anti-CMVIgG > = 14 AU/ml, in accordance with manufacturer's instructions. Percutaneous liver biopsy were performed using Tru-cut biopsy needle by the same expert in all cases. Before liver biopsy we ensured normal vital parameters, normal platelet counts, normal coagulation profile and no cystic lesion in the liver in Ultra-sonogram (USG). Informed written consent was taken from each parent or legal guardian.

Statistical Analysis: Statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) software (version 23.0; SPSS, Inc.). Categorical variables were presented as frequencies and percentages in tables and graphs; continuous variables were expressed as means and standard deviation. Correlations were assessed with the Pearson's correlation coefficient. A p-value of less than 0.05 was considered statistically significant. The approval of the research protocol was obtained from the Departmental Review Board of BSMMU.

RESULTS

Total 48 histologically confirmed BA infants associated with CM-IgM and CM-IgG positive cases were studied. Mean age of infants was 3.37±1.45 months, where 34 (70.8 %) were male and male-female ratio was almost 2.5:1.

Table I contains the distribution of clinical features of cytomegalovirus IgM and cytomegalovirus IgG positive BA. Among the cases 28 (58.3%) were in age group 2 to 3

months, 4 (8.4%) were <2 -3 months, 16 (33.3%) were >3 months at the date of admission. Among 48 cases. Regarding birth history 31 (64.6%) infants were delivered by lower uterine caesarian section (LUCS) and rest 17 (35.4%) were normal delivery. Normal birth weight with appropriate gestational age (AGA) were found in 32 (66.7%) infants and 16 (33.3%) had low birth weight. Among the infants 46 (95.8%) were term and rest 2(4.2%) were preterm. Infants mean age of appearance of jaundice was 7.67±10.98 days, where jaundice was traced before 7 days in 29 (60.4%) cases, within 7-14 days in 11 (22.9%) and after 14 days in 8 (16.7%) cases. Presence of intermittent pale stool was detected in 35 (73%) cases and the remaining had persistent pale stool 13 (27%). History of maternal fever and rash were detected in 4 (8.3%) cases. Feature of coagulopathy was present in 12 (25%) cases, where liver was palpable in 40 (83.3%) and spleen was palpable in 24 (50%) cases.

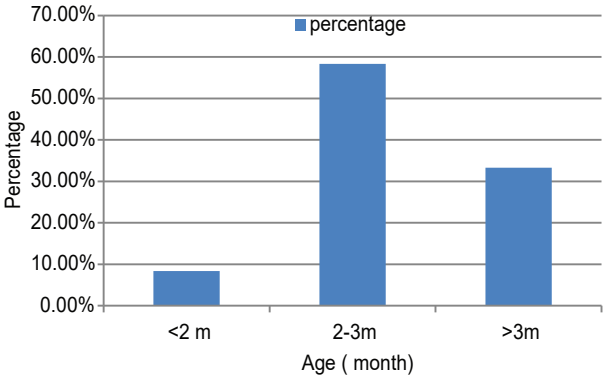

Figure- 1: Age distribution of studied subjects (n=48)

Figure 1 represents the age distribution of studied subjects, here 58.3% infants were in 2 to 3 months of age group, 8.4% were <2 months and 33.3% were >3 months of age at the time of admission.

Table- I: Clinical Features of CMV IgM & IgG positive Biliary atresia (n=48)

Clinical variables	Observations	Frequency	Percentage
Age group	<2 month	4	8.4
	2-3 month	28	58.3
	>3month	16	33.3
Sex	Male	34	70.8
	Female	14	29.2
Birth weight	Appropriate for gestational age (AGA)	32	66.7
	Low birth weight(LBW)	16	33.3
Birth history	Normal vaginal delivery (NVD)	17	35.4
	Lower uterine caesarian section(LUCS)	31	64.6
	Term	46	95.8
	Preterm	2	4.2
Jaundice onset	< 7 days	29	60.4
	7-14 days	11	22.9
	>14 days	8	16.7
Pale stool	Persistent	13	27.0
	Intermittent	35	73.0
Feature of coagulopathy		12	25
H/o maternal fever & or rash		4	8.3
Liver (palpable)		40	83.3
Spleen (palpable)		24	50.0
Total		48	100

Table II shows hematological and biochemical parameters of BA with CMV IgM and CMV IgG positive cases at the time of diagnosis. Mean hemoglobin level, mean ESR, mean platelet count and mean total WBC count were 9.4 ±1.53, 28.2±18.81, 305×10^9/L and 15.16×10^3/L respectively. Mean total and direct bilirubin was 11.89±4.07 and 8.27±3.42 respectively. Mean alanine transaminase (ALT) was 162.67±103.09, mean Gamma-glutamyl transpeptidase (GGT) was 669.46±543 and mean INR was 2.44±2.54. Mean CMV IgM and CMV IgG titer were 50.84±33.09 and 82.40±53.79 respectively.

Table- II: Hematological & biochemical parameters subjects with CMV IgM and CMV IgG positive Biliary atresia (n=48)

Hematological & Biochemical parameters	Mean	±*SD*
Hemoglobin (gm/dl)	9.4	1.53
ESR	28.2	18.81
Total count WBC[c] (×10^3 /L)	15.16	7.96
Platelet[c] (×10^9/L)	305	350
Total bilirubin (mg/dl	11.89	4.07
Direct bilirubin (mg/dl)	8.27	3.42
Alanine transaminase (IU/L	162.67	103.09
Gamma-glutamyl transpeptidase (IU/L)	669.46	543.57
International normalised ratio (INR)	2.44	2.54
Cytomegalovirus IgM	50.84	33.09
Cytomegalovirus IgG	82.40	53.79

Table III states distribution of patient by INR, level <1.2 in 24 (50%), 1.2-1.5 in 9 (19.2%) and >1.5 in 15 (30.8%) cases.

Table- III: Distribution of patient by International normalised ratio (INR) level (n=48)

INR	Frequency	Percentage
<1.2	24	50.0
1.2-1.5	9	19.2
>1.5	15	30.8
Total	48	100

Table IV shows the correlation between serum level of CMV IgM, CMV IgG titers including clinical and biochemical parameters. Serum CMV IgM titers were correlated with total bilirubin (r=-0.457; p=0.028), and direct bilirubin (r = -0.488; p=0.018). Serum CMV IgG titers were correlated with age (r = -0.530; p=0.001) and INR (r=0.593; p=0.005). Alanine transaminase (ALT) and Gammaglutamyl transpeptidase (GGT) were not significant correlation with CMV IgM, CMV IgG titers.

Table- IV: Correlation of serum CMV IgM and IgG titer with age; various biochemical parameters of CMV IgM and IgG positive titer with Biliary Atresia (n=48)

Clinical & Biochemical parameters	Cytomegalovirus IgM titer		Cytomegalovirus IgG titer	
	r-value	p-value	r-value	p-value
Age	-.301	0.153	0.5300	0.011
Total bilirubin	.457	0.028	.111	0.622
Direct Bilirubin	.488	0.018	.142	0.527
Alanine transaminase (ALT)	.096	0.656	.183	0.415
Gammaglutamyl transpeptidase (GGT)	0.057	0.806	-.092	0.698
International normalised ratio	-.279	0.198	.593	0.005

Correlations were assessed using Pearson's correlation coefficient.

DISCUSSION

Biliary atresia (BA) is a rare disease, but incidence of biliary atresia (BA) is 25.8% in our country.[15] Prevalence of cytomegalovirus (CMV) infection is higher (25.4%) in biliary atresia (BA) infant than congenital cytomegalovirus (CMV) infection in general population.[13] As a tertiary care center there is a group of biliary atresia (BA) patient in our institute who are CMV IgM & IgG antibody positive & they are sufficient in number for the study purpose. Still now there is controversy whether cytomegalovirus (CMV) infection has a role in the pathogenesis of biliary atresia or isolated pathology as clinical evidence is poor.[16] so it is reasonable to study biliary atresia (BA) associated with cytomegalovirus (CMV) from a different point of view. Mean age of the studied cytomegalovirus IgM & IgG positive biliary atresia (BA) patients at admission was 3.37±1.45 month. In a previous study conducted at the same center showed mean age was 3.3 months in biliary atresia (BA) infant.[17] Another study mention

cytomegalovirus IgM positive biliary atresia (BA) patient were older 70 days at Kasai Porto-enterostomy (KPE) than cytomegalovirus IgM negative biliary atresia (BA).[14] Biliary atresia is common in female infant as well as cytomegalovirus IgM positive biliary atresia(BA) is also reported to be common in female infants.[14,18] Biliary atresia (BA) is common (61.6%) in male infant in previous study at the same center conducted in 2005.[17] Now there is also male predominance 34(70.8%) in cytomegalovirus IgM & IgG positive Biliary atresia. There may be symptom of maternal Primary cytomegalovirus (CMV) infection during pregnancy period including fever, headache, malaise, pharyngitis, hepatosplenomegaly, lymphadenopathy, arthralgias, and rash causing congenital cytomegalovirus (CMV) infection. In our study history of maternal fever & rash was present in only 4(8.3%) indicating that there may be perinatal CMV infection rather than congenital infection**.** However, this symptom also may present in Epstein Barr virus (EBV) infection.[19] Infant with congenital CMV infection present with symptoms of Jaundice, petechiae, chorioretinitis, and cataracts hepatosplenomegaly intracranial calcifications, microcephaly, ventriculomegaly intrauterine growth restriction, pericardial effusion, premature or small for gestational age, hearing loss. Our patient had no feature of congenital CMV infection except jaundice & hepatosplenomegaly. Liver is palpable in 40(83.3%) child which is common feature of biliary atresia & CMV infection. Spleen was palpable in 24 (50%) child may be as part of CMV infection because there is no evidence of accessory spleen or double spleen evident by ultrasonography & no feature of biliary cirrhosis. Hearing loss is the most common sequelae associated with congenital CMV infection. Hearing assessment done in all of our patient reporting no hearing impairment. This finding also excludes congenital CMV infection. 32(66.7%) of our child had appropriate for gestational age (AGA) & 16(33.3%) had low birth weight, 46(95.8%) were term, 2(4.2%) were preterm baby. Congenital CMV infection could be excluded by doing CMV DNA or IgM within 3 weeks of delivery, which was not possible for our study due to late presentation.[19] Biliary atresia associated with positive CMV IgM differs from other types of biliary atresia by late onset clinical manifestations. The patient of CMV IgM positive BA appears healthy during birth, but obstructive cholestasis is developed after the second week of life & gradually progress over time.[20] In our study mean age of onset of jaundice was 7.67±10.98 days. Jaundice started to appear before 7 days in 29 (60.4%) cases, within 7-14 days in 11(22.9%) cases & after7 days in 8(16.7%) cases. Feature of coagulopathy was present in 12(25%) cases. In a previous study CMV IgM positive group BA had pale stool in 47.4% cases, pigmented stool 52.6% cases. Presence of pigmented stool was more common (35, 73%), the remaining had persistent pale stool (13, 27%) in this study. As for liver function, the median levels of alanine transaminase (ALT), total bilirubin, direct bilirubin, Gamma-glutamyl transpeptidase (GGT) were 140, 9.5, 6.08, and 377 respectively.[21] In Our CMV IgM & IgG positive BA patient mean total & direct bilirubin was 11.89±4.07, 8.27±3.42 respectively, mean alanine transaminase (ALT), was162.67±103.09, mean Gamma-glutamyl transpeptidase (GGT) was higher 669.46±543, mean INR was 2.44±2.54. Different study uses different test method to detect presence of CMV infection like serum CMV IgG, CMV IgM, and CMV-DNA, CMV-pp65. We used CMV IgG & CMV IgM, because urinary CMV DNA was not available during study period in our institute. However if number of virus less than the limit of PCR sensitivity, CMV infection cannot be ruled out by CMV DNA. Moreover in case of persistent infection intermittent shedding of virus occur.[7] Mean CMV IgM, CMV IgG titer were 50.84±33.09, 82.40±53.79 respectively in our study There is positive clinical correlation between age & serum level of CMV IgG (r=-0.530; p=0.001) & statistically significant indicating increase in age would increase titer of IgG. Serum CMV IgG titers were also positively correlated with INR (r=0.593; p=0.005) levels. So increase titer level associated with increased INR level or coagulopathy. Serum CMV IgM titers were positively correlated with total bilirubin(r=-0.457; p=0.028) & direct bilirubin (r=-0.488; p=0.018). Increase in serum CMV IgM titers would increase total & direct bilirubin level. Previous studies suggested that newborn infants with congenital Cytomegalovirus infection could be identified by CMV IgM reactivity and elevated levels of CMV IgM correlated with the disease severity. Manifestations of CMV infection reflect the level of virus replication as well as the end-organ involvement.[22]

CONCLUSIONS

This study concluded that about three fourth of CMV IgM and IgG positive BA patient presented with pigmented or intermittent pale stool, one fourth patient had feature of coagulopathy. Most of the infants were term baby and half of them had splenomegaly. Study fiends that an increase in age would increase titer of IgG and increase in titer

associated with increase coagulopathy. Elevated levels of CMV IgM titer correlated with bilirubin level or cholestasis. However, multi-center based study with large sample size may need to reach a consensus.

LIMITATION

It was a single center study with small sample size. Though real - time PCR is more sensitive and specific for detection of primary CMV infection than CMV IgM and CMV IgG.

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