The Association Between Biliary Atresia and Cytomegalovirus Hepatitis

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Abstract

Introduction: Biliary atresia (BA) is a disease characterized by a biliary obstruction of unknown origin. Viral agents have been proposed in the aetiology of BA such as cytomegalovirus (CMV). This virus also considered as a one of agents that can infect the liver and cause hepatitis. The aim of this study was to determine the role of CMV in children with both chronic hepatitis (negative for hepatitis B and C) and have biliary atresia in the same time. **Material and Methods:** A retrospective study done on 13 liver tissue paraffin blocks of children with chronic hepatitis (negative for hepatitis B and C) and biliary atresia (extra and intra). The diagnosis was based on the presence of HCMV protein (pp65) by using immunohistochemistry. **Results:** Immunohistochemistry for pp65 showed the liver tissue blocks were positive for 10 cases (76.9%).The mild inflammation and moderate fibrosis were the highest among the cases. **Conclusion:** CMV is one of the important viruses that can causes hepatitis in infants (whom are negative for hepatitis B and C), also this virus has significant role in pathogenesis of biliary atresia.

Key words: Cytomegalovirus, biliary atresia, hepatitis, immunohistochemistry, infants.

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Introduction

Biliary atresia (BA) is a disease characterized by a biliary obstruction of unknown origin that presents in the neonatal period and it is the most frequent surgical cause of cholestatic jaundice in neonates, also it is a severe hepatobiliary disease in infancy characterized by a progressive, fibro-obliterative process affecting extrahepatic as well as intrahepatic bile ducts, leading to early liver cirrhosis[1]. The cause of BA remains unknown. Theories on pathogenesis include genetic predisposition, abnormal morphogenesis, vascular abnormalities, exposure to environmental toxins, viral infection, and autoimmune mediated bile duct destruction[2].

Multiple viruses including hepatitis B, human papillomavirus, Epstein-Barr virus, cytomegalovirus (CMV), rotavirus and reovirus have been proposed in the etiology of BA.[3,4,5,6]. Cytomegalovirus (CMV) is a member of the Herpesviridae[7]. CMV infection is acquired either in the perinatal period and infancy or in adulthood through sexual contact, organ transplantation or blood transfusion[8]. CMV considered as a one of agents that can infect the liver and cause hepatitis[9]. The virus can replicate in both hepatocytes and cholangiocytes, it could directly induce injury in the liver and bile duct system, and induce immune damage in infected cells, revealing inclusion bodies in hepatocytes and vascular epithelial cells, especially in epithelial lining cells of bile duct[10]. This study aimed to determine the role of CMV in children with both chronic hepatitis

(negative for hepatitis B and C) and have biliary atresia (extra and intra) in the same time.

Materials and Methods

A retrospective study done on 13 liver tissue paraffin blocks of patients admitted to Gastroenterology and Hepatology Teaching Hospital and Children Welfare Teaching Hospital (Medical City- Baghdad). Specimens collection was done in the period from February 2014 to August 2014, for liver biopsies that were done during the period from 2008 to 2014 for children suffering un explained hepatitis (serologically negative for hepatitis B and C) with biliary atresia (9 cases extra and 4 cases intra). Patients records were revised for: Age, sex, clinical manifestations. The diagnosis based on the presence of CMV protein (pp65) by using immunohistochemistry (IHC). Immunohistochemical staining was performed on paraffin blocks after sectioned (4 µm). We dewaxed the slides in xylene followed by rehydration via a descending ethanol series (90%–50%). The slides were placed in the antigen retrival solution (Sodium citrate buffer, PH 6.0) in water bath 95Cº for 5 min. Sections were blocked for endogenous peroxidase (3% H2O2, for 15 min at room temperature). Drops of protein blocks were added to slide s (15 min at room temperature). Monoclonal antibody anti CMV pp65 (dilution 1:150, code number: ab49214, Abcam, USA) was added to slides and incubated at 37 Cº for 1 hr, then placed in 4Cº overnight. For visualization, we used a horseradish peroxidase detection system. Finally, positive cells were visualized with the chromogendiaminobenzidine (DAB). After counter staining them with hematoxylin. Dehydration was done and sections were mounted with Disteren Plasticizer Xylene (DPX) and covered by coverslips and examined under light microscope. Positive control slide of Newcomer supply company (USA), this slide are positive for CMV proteins. Single experienced histopathologist revised the biopsies tissue for grading for inflammation and staging for fibrosis. The grading of inflammation and staging of fibrosis were determined according to Knodell histological activity index as follows; inflammation : Nil (≤2), Mild (3-6), Moderate (7-11) and severe (≥12) while the fibrosis: Nil (1), Mild (2), Moderate (3) and Severe (≥4).

Statistical analysis: The statistical analysis of this study performed with the statistical package for social sciences (SPSS) 19.0 and Microsoft Excel 2013. Categorical data formulated as count and percentage. Chi-square test was used to describe the association of these data. The lower level of accepted statistical significant difference is below 0.05.

Results

Positive IHC for CMV pp65 was 10 cases (76.9%) as shown in Table 1 and Figure 1. The mean age of patients was (3.8) months with median (3) months (the age range from 2-14 months) and ten (76.9%) of thirteen were younger than 6 months of age. There was a ratio of nine males to four females (Table 1).

From records, clinical symptoms at presentation included jaundice 13 (100%), followed by pale stools 6 (46.1%) and hepatosplenomegaly 4 (30.7%).

According to histopathological reports, there were grading for inflammation and staging for fibrosis (Figure 3 and 4). There were 6 of 8 (75%) of cases with mild inflammation were positive for CMV pp65IHC. There three out of six (50%) of cases with moderate fibrosis were positive for CMV IHC. There were 10 (76.9%) cases showed bile duct proliferation (Figure 2) and 6 (46.1%) with giant cell hepatitis (Figure 3). There were also 6 (46.1%) cases showing bile thrombi in their liver histology (Figure 4).

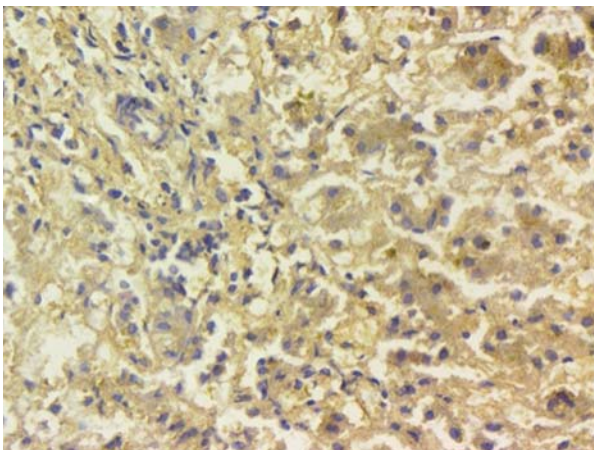


Fig 1: Positive result of CMV pp65 IHC in liver tissue showing brown cytoplasmic and perinuclear stain (IHC stain, 40X)

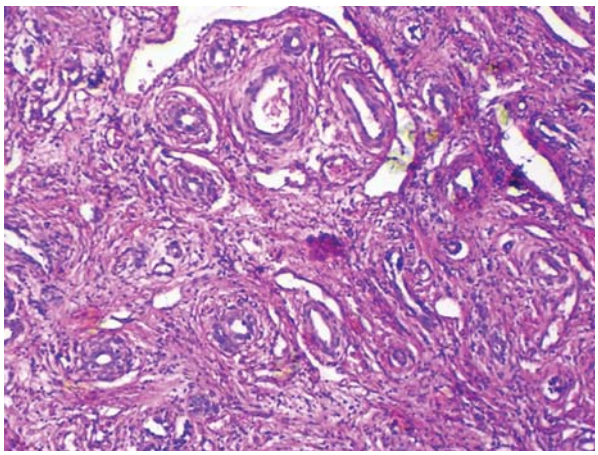


Fig 2: Section showed prominent bile duct proliferation with extensive fibrosis expanding the portal area (H&E stain, 10X)

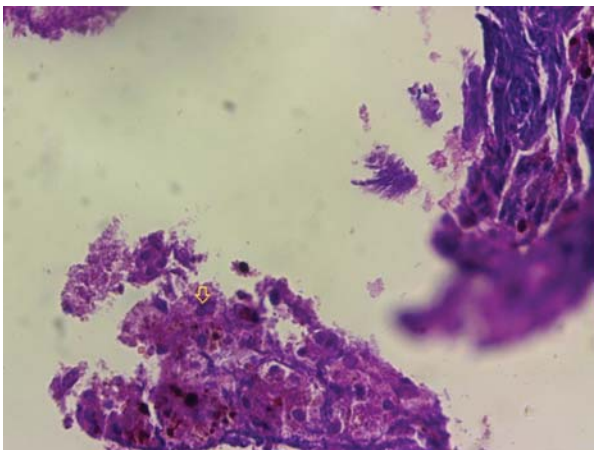


Fig 3: A sheet of hepatocytes with gigantic nuclei (H&E stain, 40X)

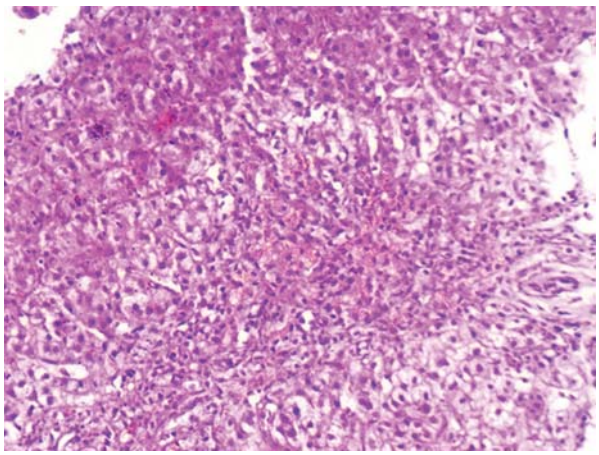
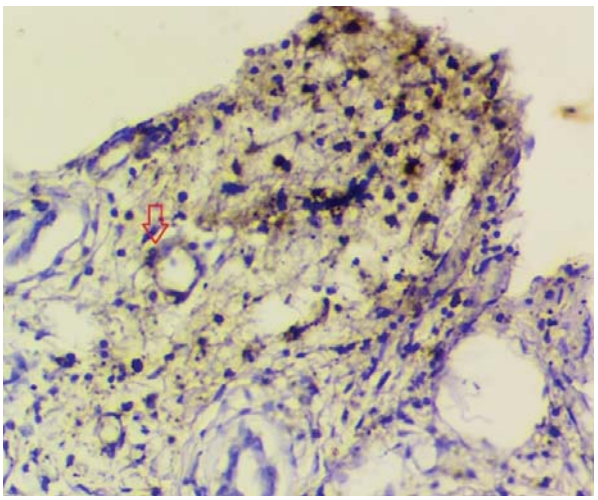


Fig 4: Moderate cholestasis, both hepatocellular and canalicular with bile thrombi and associated with necroinflammatory changes in related to it. Portal area showed paucity of bile duct with prominent bile duct proliferation (H&E stain, 40X).



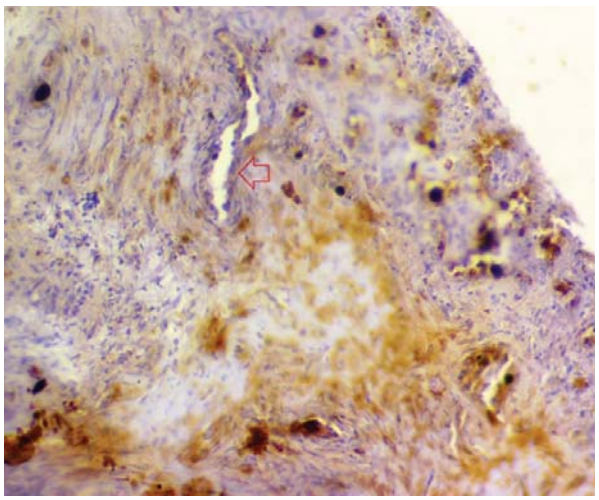


Fig 5 and 6: Bile duct cholangiocytes staining positive of CMV pp65 with perinuclear and cytoplasmic pattern of staining (fig 5 20X, fig 6 40X IHC stain)

Table 1: Characteristics of patients with CMV pp65 IHC result

CMV pp65 IHC	Type of BA	Signs & symptoms			Sex	Age months	Patients No.
		P. stool	HSM	Jaundice			
+ve	Extra	No	Yes	Yes	M	2.5	1
+ve	Extra	No	No	Yes	M	4.5	2
+ve	Extra	No	No	Yes	M	3	3
+ve	Extra	No	No	Yes	M	2	4
-ve	Extra	No	No	Yes	F	6	5
+ve	Extra	Yes	Yes	Yes	F	3	6
-ve	Extra	Yes	Yes	Yes	M	14	7
+ve	Extra	No	No	Yes	F	2.5	8
+ve	Extra	No	Yes	Yes	M	2	9
+ve	Intra	Yes	No	Yes	M	2.5	10
+ve	Intra	Yes	No	Yes	F	6	11
-ve	Intra	Yes	No	Yes	M	2.5	12
+ve	Intra	Yes	No	Yes	M	3.5	13

M:male, F:female, HSM:hepatosplenomegaly, P. stool: Pale stool.

Table 2: Distribution of CMV pp65 IHC according to inflammation

	CMV pp65 IHC							
P value	Positive		Negative		Total			
	%	No	%	No	%	NO		
	0%	0	0%	0	0%	0	Nil	
	75%	6	25%	2	61.5%	8	Mild	
0.102	100%	4	0%	0	30.7%	4	Moderate	Inflammation
	0%	0	100%	1	7.6%	1	Severe	
	76%	10	23.1%	3	100%	13	Total	

Table 3: Distribution of CMV pp65 IHC according to fibrosis

	CMV pp65 IHC							
P value	Positive		Negative		Total			
	%	No	%	No	%	No		
	100%	1	0%	0	7.6%	1	Nil	
	100%	2	0%	0	15.3%	2	Mild	
0.207	50%	3	50%	3	46.1%	6	Moderate	Fibrosis
	100%	4	0%	0	30.7%	4	Severe	
	80%	12	20%	3	100%	13	Total	

Discussion

CMV infection is the most frequent congenital infection worldwide and is various in its clinical manifestations[11]. Infants may acquire CMV infection from the mother as a result of intrauterine infection (congenital infection), or through contact with infected genital secretions during passage through the birth canal (perinatal infection), or postpartum through breast feeding (postnatal infection)[12].

CMV has the ability to replicate in both hepatocytes and cholangiocytes. This virus could directly induce injury in the liver and bile duct system, and induce immune damage in infected cells, revealing inclusion bodies in hepatocytes and vascular epithelial cells, especially in epithelial lining cells of bile duct[10] (Figures 5 and 6). The swollen bile duct epithelium which is the cause of the unsmooth biliary flow could lead to intra-/ extra- hepatic cholestasis[13].

In this study the detection of CMV infection was based on presence of virus protein pp65. This protein represents the largest component in virus structure[14]. The expression of this protein coincides with viral lytic replication[15], also the expression of this protein reflect the active viral infection[16].

Most of cases in this study were located in the age of less than 6 months; and this may be due to immaturity of immune system, congenital and perinatal infection[17,18]. Similar age prevalence was reported also by Soomro et al 2011[19].

The cases with mild histological inflammation were the highest among others and the cases with moderate histological fibrosis were the highest among others. Regarding inflammation, the explanation of this situation may be due to that inflammation is still in its beginning or inflammation ended from long time with significant fibrosis. While for the fibrosis, the result reflects the extent of persistent or progressive hepatic injury and the small age of patients may be do not give a chance for fibrosis to develop into severe stage[20]. Ten cases with cases with bile duct proliferation. The obstruction of bile duct leads to lack of bile flow and as the result of the pressure of bile cause bile duct proliferation[21]. Also six cases with giant cell hepatitis, it's a common histological finding in infants with neonatal cholestasis[22].

Conclusions

From current study, we conclude that CMV is one of the important viruses that can causes hepatitis in infants (whom are negative for hepatitis B and C), also this virus has significant role in pathogenesis of biliary atresia.

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