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Research Article

Prevalence of Serological Markers of TORCH Infections in Biliary Atresia and Other Neonatal Cholestatic Disorders

Abstract

Background: Viral infections, congenitally or perinatally acquired, have been associated with neonatal cholestasis. Investigators have suggested a similar link to biliary atresia (BA).

Aim: The aim of the current study is to investigate the prevalence of serological markers of congenital infections in BA and other neonatal cholestatic disorders.

Methods: This retrospective study included 94 patients with confirmed diagnosis of BA. A nearly comparable number of patients with cholestasis due to causes other than BA ($n = 91$) were also recruited and termed non-BA group. The data was retrieved from patients' records. TORCH (toxoplasma, rubella, cytomegalovirus [CMV] and herpes simplex virus [HSV] type 1 and type 2) antibodies (immunoglobulin [Ig] M and IgG) were performed in all the patients using enzyme-linked immunosorbent assay. CMV DNA was detected by polymerase chain reaction (PCR).

Results: Both groups were age and sex matched ($P > 0.05$). TORCH IgM antibodies were detected in 15.7% of all the study population (8.5% in BA group and 23% in non-BA group), of which CMV was the commonest agent. CMV IgM and CMV DNA by PCR were significantly higher in non-BA group (20.9% and 23% respectively) than in BA group (4.3% and 5.3% respectively). Toxoplasma IgM was positive in only one patient in BA group and rubella IgM was positive only in one patient in the non-BA group. HSV-1 IgM was found in a total of 4 patients; 3 in BA group and one in non-BA group while HSV-2 IgM was negative in all the patients. CMV infection was the sole incriminated agent in 13 patients (CMV hepatitis), while it was associated with other etiologies such as progressive familial intrahepatic cholestasis (5 of 29 patients), and intrahepatic biliary paucity (3 of 14 patients). Liver transaminases, prothrombin time and the frequency of growth failure were significantly higher in non-BA group.

Conclusions: TORCH IgM antibodies were detected in 8.5% of BA and in 23% of non-BA group, of which CMV was the commonest agent. In addition to CMV hepatitis, CMV infection was associated with other causes of neonatal cholestasis. For that, all cases with neonatal cholestasis should be thoroughly evaluated for other causes, even in cases with demonstrable CMV infection.

Abbreviations

BA: biliary atresia; CMV: cytomegalovirus; HSV: herpes simplex virus; Ig: immunoglobulin; IOC: intraoperative cholangiography; PCR: polymerase chain reaction; TORCH: toxoplasma, rubella, CMV, HSV

Introduction

Neonatal cholestasis is generally defined as conjugated hyperbilirubinemia that occurs in the newborn period or

shortly thereafter. Cholestasis results from diminished bile formation and/or excretion, which can be caused by a number of disorders. The term "neonatal cholestasis" is often used to refer to cholestatic liver disease that is present at birth or develops within the first few months of life, rather than referring strictly to the neonatal period. In clinical practice, these disorders usually become apparent within the first two months of life, which is the critical period for identifying infants with biliary atresia (BA) [1,2].

BA is the most common disease in this age group and

consistently has accounted for one third of all neonatal cholestasis and more than 90% of obstructive cholestasis cases. The only available treatment is early surgical intervention with Kasai portoenterostomy [3,4]. If portoenterostomy is not successful or not performed, liver transplantation is the only life-saving alternative [5]. Congenital infections caused by TORCH (toxoplasma, rubella, cytomegalovirus [CMV] and herpes simplex virus [HSV] type 1 and type 2), were also incriminated as an etiological factors for neonatal cholestasis [6].

The etiology of BA is poorly understood. There is controversy about the etiological role of viruses. The studies have implicated reoviruses, rotaviruses and CMV [7]. The initial event may be a viral infection, which targets the biliary epithelium [8]. This is followed by activation of immune cells and release of proinflammatory cytokines [9] and adhesion molecules [10,11] that perpetuates the injury and causes biliary destruction, leading to the atresia phenotype [12].

CMV hepatitis may present with cholestasis and clay colored stool, a presentation which is similar to that of BA [13]. The association between BA and CMV has been investigated and described by different groups [14–16]. It was reported that CMV hepatitis may proceed to progressive hepatic fibrosis and cirrhosis [17] and the establishment of CMV infection in infants with cholestasis should not deter the search for other etiologies of cholestasis [18].

It is of importance to understand the magnitude of congenital infections as a contributor to neonatal cholestasis. For that, we aimed to investigate the prevalence of serological markers of TORCH infections among infants with BA and other neonatal cholestatic disorders.

Patients and Methods

Study population

This retrospective study included 94 patients with BA attending the department of Pediatric Hepatology, Gastroenterology and Nutrition over a period of three years between May 2011 and June 2013. The data was retrieved from patients' records. A nearly comparable number of patients with cholestasis due to causes other than BA ($n = 91$) were recruited within nearly the same time period and termed non-BA group. During retrieval process, those with incomplete data or those without liver biopsy were excluded from the study. A written informed consent was not needed due to the retrospective nature of the study. The study was approved by the Research Ethics Committee of National Liver Institute, Menofiya University.

Etiological diagnosis

After full history taking, thorough clinical examination and routine (laboratory, ultrasonography and liver biopsy) investigations, the diagnosis of BA was confirmed by intraoperative cholangiography (IOC) prior to corrective surgery (Kasai operation). In the non-BA group, clinical evaluation together with a set of specific laboratory tests

according to the expected etiology, liver biopsy and negative IOC in some patients, the diagnosis of BA was ruled out in the patients of this group. Diagnoses in the non-BA group were as presented in Table 1.

Viral and Toxoplasma markers

TORCH antibodies (immunoglobulin [Ig]M and IgG) were performed in all the patients, using enzyme-linked immunosorbent assay according to the manufacturer instructions; Toxoplasma antibodies (Pishtaz Teb Zaman Diagnostics, Tehran, Iran), HSV-1, HSV-2, CMV and rubella antibodies (all from Diapro diagnostic bioprobes, Milano, Italy). CMV-DNA polymerase chain reaction (PCR): By COBAS AMPLICOR monitor test, version 2.0, COBAS AMPLICOR Analyzer, Roche [19]. The detection limit of the kit was 200 copy/ml.

Statistical methods

Descriptive results were expressed as mean \pm standard deviation (mean \pm SD) or number and percentage. For quantitative data, significance was tested either by independent sample *t*-test or Mann-Whitney *U*-test according to the nature of the data. For qualitative data, significance was tested by Chi-square test. Results were considered significant if *P*-value < 0.05 . Statistical analysis was performed using SPSS statistical package version 13 (SPSS Inc, Chicago, IL, USA).

Results

Study population's characteristics

This study included 185 individuals divided according to the diagnosis into BA group ($n = 94$) and non-BA group ($n = 91$). Baseline demographic, clinical and laboratory characteristics were comparable in both BA and non-BA groups except for clay stool, platelet count, and gammaglutamyl transpeptidase which were significantly higher in BA than in the non-BA group. On the other hand, transaminases and prothrombin time were

Table 1: Diagnoses in the non-BA group

Diagnosis	<i>n</i>
Idiopathic neonatal hepatitis	18
Progressive familial intrahepatic cholestasis *	29
Galactosemia	6
Intrahepatic biliary paucity *	14
Tyrosinemia	1
Cystic fibrosis	1
Caroli disease	1
Mitochondrial hepatopathy	1
Congenital hepatic fibrosis	1
Cholodocal cyst	1
Alagille syndrome	3
Cytomegalovirus hepatitis *	13
Glycogen storage disease type IV	2

* Cytomegalovirus DNA was positive in progressive familial intrahepatic cholestasis ($n=5$), intrahepatic biliary paucity ($n=3$) and cytomegalovirus hepatitis ($n=13$)

significantly higher in the non-BA group. Of importance, 27 (29.7%) of cholestasis group had growth failure compared to only 9 (9.6%) of BA group ($P = 0.001$) (Table 2).

The occurrence of toxoplasma and viral markers in the studied patients

Among the studied serological makers, CMV IgM and CMV DNA were the most frequent (12.4% and 14.1% respectively) in the study population. IgG antibodies of all infections were detected in the majority (from 50.8% to 82.2%) of patients except for that of toxoplasma which was detected in the minority (37.8) of patients (Table 3).

Looking at BA and non-BA groups individually, the occurrence of viral markers was comparable in both groups except for CMV IgM (which was confirmed by PCR for CMV DNA) that was significantly higher in Non-BA. The occurrence of IgG antibodies of the studied infections was more frequent than IgM antibodies and was comparable in both groups ($P > 0.05$). Generally, CMV represents the high frequency in both groups (Table 3).

CMV infection was the sole incriminated agent in 13 patients (CMV hepatitis), while it was associated with other etiologies as BA ($n = 4$), progressive familial intrahepatic cholestasis (5 of 29 patients), and intrahepatic biliary paucity (3 of 14 patients) (Table 1). Toxoplasma IgM was positive in only one patient in BA group and rubella IgM was positive only in one patient in the non-BA group. HSV-1 IgM was detected in a total of 4 patients; 3 in the BA group and one in the non-BA group while HSV-2 IgM was negative in all the patients.

Table 2: Demographic, clinical, and laboratory characteristics of the studied patients

Characteristics	BA ($n = 94$)	non-BA ($n = 91$)	P-value
Age (days)	60.86 ± 15.77	67.30 ± 26.78	0.087
Male n (%)	42 (44.7%)	50 (54.9%)	0.163
Growth failure	9 (9.6%)	27 (29.7%)	0.001
Hepatomegaly n (%)	94 (100%)	87 (95.6%)	0.057
Splenomegaly n (%)	66 (70.2%)	58 (63.7%)	0.053
Clay-colored stool	92 (97.9)	32 (35.2%)	<0.0001
Total bilirubin (mg/dl)	11.27 ± 3.91	11.64 ± 6.54	0.529
Direct bilirubin (mg/dl)	7.20 ± 2.97	7.67 ± 4.23	0.678
Alanine transaminase (U/L)	134.51 ± 101.99	204.21 ± 173.47	0.001
Aspartate transaminase (U/L)	230.57 ± 156.03	367.02 ± 313.69	0.001
Total proteins (g/dl)	5.46 ± 0.72	5.36 ± 0.91	0.33
Albumin (g/dl)	3.41 ± 0.54	3.29 ± 0.63	0.221
Alkaline phosphatase (U/L)	519.07 ± 231.33	597.33 ± 368.04	0.304
gamma-glutamyl transpeptidase (U/L)	682.66 ± 558.39	231.26 ± 252.83	<0.0001
Prothrombin time (seconds)	12.62 ± 1.23	13.4 ± 1.98	0.009

Table 3: Occurrence of TORCH markers in the studied patients

Viral markers	All patients ($n = 185$)	BA ($n = 94$)	non-BA ($n = 91$)	P-value
Toxoplasma IgM	1 (0.5)	1 (1.1)	0.0	1.0
Toxoplasma IgG	70 (37.8)	35 (37.2)	35 (38.5)	0.863
HSV-1 IgM	4 (2.2)	3 (3.1)	1 (1.1)	0.621
HSV-1 IgG	97 (52.4)	46 (48.9)	51 (56)	0.333
HSV-2 IgM	0.0 (0%)	0.0 (0%)	0.0 (0%)	–
HSV-2 IgG	45 (24.3)	23 (24.5)	22 (24.2)	0.693
Cytomegalovirus IgM	23 (12.4)	4 (4.3)	19 (20.9)	0.001
Cytomegalovirus IgG	152 (82.2)	75 (79.8)	77 (84.6)	0.391
Cytomegalovirus DNA	26 (14.1)	5 (5.3)	21 (23)	0.001
Rubella virus IgM	1 (0.5)	0.0	1 (1.1)	0.492
Rubella virus IgG	94 (50.8)	47 (50)	47 (51.6)	0.823
Total IgM positive cases	29 (15.7)	8 (8.5)	21 (23)	0.006

HSV: herpes simplex virus; P-value represents significance between BA and Non-BA group

Discussion

Viral infections, congenitally or perinatally acquired, have been associated with neonatal cholestasis. In 1974, Benjamin Landing a pediatric pathologist proposed that BA as well as choledochal cyst and neonatal hepatitis represent varied manifestations of a single basic disease process and coined the term infantile obstructive cholangiopathies to describe these entities [20].

We separated the current study population into BA and non-BA groups. The reason for this separation is not to point out the discriminative laboratory or clinical parameters between both groups. We have already covered this issue extensively in our previous studies [10,11,21,22], so it will not be discussed here. But as BA is the main etiologic cause of neonatal cholestasis [23] and as CMV has been proposed as a possible etiologic agent for BA [18], this separation seemed logical.

In the current study, IgM antibodies for TORCH infections were detected in a total of 29/185 (15.6%) of patients. The prevalence of IgM antibodies was significantly higher in non-BA group compared to BA group (23% vs. 8.5%; $P = 0.006$) with CMV showing the highest frequency. CMV IgM and CMV DNA by PCR were significantly higher in non-BA group (20.9% and 23% respectively) than in BA group (4.3% and 5.3% respectively). On the other hand, there was no significant statistical difference regarding the other studied markers. Similar to our results, Quack et al. [24], reported that TORCH infections constitute 22% cases of neonatal cholestasis, of which CMV was the commonest agent. The high frequency of IgG antibodies of TORCH infections may represent transplacental transfer from the mother in most cases, which confer passive immunity to the fetus [25]. Some patients with positive CMV DNA were negative for CMV IgM. A possible explanation is that IgM had not yet been produced or that the infants had been infected for a long time that no viral IgM production persisted [26].

Many studies investigating the role of CMV infection in the pathogenesis of BA reported conflicting results. Tarr et

al [18] reported that 5 out of 23 patients (24%) were found to be positive for CMV by analyzing liver histology and CMV IgM. Oliveira et al [27] found positive CMV IgM in 28.5% of patients with BA or choledochal cysts. Similarly, Fischler et al [26] showed a higher prevalence of CMV antibodies in mothers of BA infants, and CMV DNA was present in livers of 9 /18 (50%) of BA infants. Contrarily, a Canadian group could not demonstrate CMV DNA in bile duct remnants removed from 12 children with BA at the time of the Kasai portoenterostomy. CMV cannot be conclusively excluded as an etiology of BA in selected patients, but it does not appear to be involved in the large majority of cases [28].

The positivity of CMV IgM or DNA does not necessarily indicate that it is the cause of the cholestasis, but it implies that the virus may have influenced the severity of the original pathology [29]. This may explain the significantly higher levels of transaminases and prothrombin time in non-BA group compared to BA group ($P = 0.001$ and 0.009 respectively). Furthermore, 27 (29.7%) of non-BA group had growth failure compared to 9 (9.6%) in BA group ($P = 0.001$). This may be explained by the fact that early intrauterine CMV infection induces disturbances in intrauterine and postnatal growth [29]. On the other hand, the majority of BA patients appear entirely well-nourished and well-developed during the first 4-6 weeks of life [30].

In the current study, CMV DNA was detected in 3 infants of 12 (25%) with intrahepatic biliary paucity. Zabiegaj-Zwick et al [16] reported that CMV has been associated with intrahepatic bile duct destruction and duct paucity. Whether it is the cause of this pathology or just exaggerating the original disease; remains to be elucidated.

A recent study by Zani et al [31] hypothesized that CMV IgM-positive BA is a separate clinical entity compared to CMV IgM-negative BA with unfavorable response to Kasai portoenterostomy. CMV infection was not always the only factor causing cholestatic disease and it must be emphasized that all cases with neonatal cholestasis should be thoroughly evaluated for other causes, even in the case of demonstrable CMV infection, so that other treatable disorders such as BA are not missed [18].

Our results showed that, in addition to all the patients with CMV hepatitis, CMV DNA was detected in some patients with BA, progressive familial intrahepatic cholestasis, and intrahepatic biliary paucity. Interestingly, we recently reported that CMV hepatitis, and progressive familial intrahepatic cholestasis can have a clinicopathological features similar to that of BA [32].

The notion that CMV may cause BA is more complex than it seems. Fischler et al [26] questioned the role of CMV even when detected in liver tissue. One important question was whether the detected CMV DNA really originated from liver cells or simply represented a carry-over from peripheral leukocytes in the same liver specimens; the latter possibility cannot be ruled out. On the other hand, CMV-induced immune mechanisms may affect the liver, even if virus is no longer demonstrable in liver tissue.

Recent studies suggested a number of genes [33-38] and toxins [39,40] that may be incriminated in the development of the BA phenotype. These findings have opened the horizon for novel etiopathogenic mechanism of BA and narrowed the spectrum of cases that were imputed to viral infection.

In conclusion, TORCH IgM antibodies were detected in 8.5% of BA and in 23% of non-BA group, of which CMV was the commonest agent. CMV may be incriminated in only few cases of BA. All cases with neonatal cholestasis should be thoroughly evaluated for other causes of cholestasis, even in cases with demonstrable CMV infection.

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