

## Role of Some Viral Infections in Neonatal Cholestasis

<sup>1</sup>Osama T Amer, <sup>1</sup>Hanan A Abd El-Rahma, <sup>1</sup>Laila M Sherief, <sup>1</sup>Hani F Hussein, <sup>2</sup>Ayman F Zeid and <sup>3</sup>Azza M Abd El-Aziz

<sup>1</sup>Pediatric and <sup>2</sup>Radiology Departments, Faculty of Medicine, Zagazig University and <sup>3</sup>Microbiology Department, National Liver Institute, Monefia University, Egypt.

Viral infection is one of the postulated causes of neonatal cholestasis. In addition to earlier reports on the association of viral infection and intrahepatic cholestasis (IH), recent studies have suggested a similar link to extra hepatic biliary atresia (EHBA). The aim of this work was to evaluate the role of some viral infections in neonates presenting with cholestasis to the Neonatology Ward of Zagazig University Hospitals. Sixty-two neonates were included in the study (44 cholestatic neonates and 18 apparently healthy neonates as the control group). All neonates were subjected to full history taking and complete physical examination. Laboratory investigations included CBC, liver function tests, bleeding profile, blood cultures, abdominal ultrasound and detection of HBsAg and serum IgM antibodies against certain viruses (CMV, Reovirus III, HSV I, HSV II, Rubella virus) using ELISA. Radionuclide cholescintigraphy was performed for patients only. The study revealed that cholestatic neonates were significantly associated with dark urine, pale stool and hepatomegaly compared with the control ( $P < 0.004$ ,  $P < 0.001$ ,  $P < 0.008$ , respectively). Quantitation of IgM antibody titre using ELISA revealed significantly higher levels of serum anti-CMV IgM and anti-Reovirus III IgM in cholestatic than in the control groups. No significant differences were found in levels of anti-HSV I, anti-HSV II or anti-rubella antibodies between cholestatic and control groups. HBsAg was negative for all neonates; cholestatic and control. Lastly, no significant differences were found between neonates with EHBA (7 cases) and Intrahepatic cholestasis (37 cases) regarding anti -CMV IgM or anti -Reo III IgM. It can be concluded that CMV and Reovirus type 3 infections of the neonates are associated with the development of cholestatic disorder, not only due to IH cholestasis but also due to the production of EHBA.

**V**iral infection is one of the most prevalent causes of neonatal cholestasis. It may be related to the development of neonatal hepatitis, paucity of interlobular biliary ducts (PILBD), as well as extra hepatic biliary atresia (Fischler et al., 2001). Infection may occur by trans-placental spread, via the ascending route from vaginal or cervical secretions into amniotic fluid, from swallowed contaminated fluids (blood, urine), during delivery or from breast milk (Hicks et al., 2001). Although classically the congenital infection have thought of the acronym TORCH, the recognition of other important agents causing vertically transmitted infections makes this term archaic (Burchett, 2003).

Cytomegalovirus (CMV) is a DNA virus, which belongs to Herpes viridae family. It derives its name from the cytopathology of infected cells, which is characterized by intracellular and cytoplasmic inclusions

(Taylor, 2003). Conjugated hyperbilirubinaemia in CMV infected neonates is not only due to intrahepatic cholestasis (hepatitis and PILBD) but also may be attributed to the development of EHBA. (Tarr et al., 1996).

Herpes simplex virus (HSV) is a DNA virus from the Herpes group. Two types of HSV are known; HSV-I and HSV-II. HSV-II is the predominant cause of neonatal disease, but both types produce clinically indistinguishable syndromes (Domiaty-Saad et al., 2000). Infection of the newborn occurs as a result of intra partum transmission due to active shedding of virus from the cervix or vulva at the time of delivery (85%). In utero infection has been documented but uncommon (5%). Postnatal transmission through oropharyngeal route from parents, hospital personnel or other contacts may occur (10%) (Stoll and Weismann, 1997). HSV produces neonatal cholestasis through

hepatitis even without other manifestations, but no reports suggest a link between HSV and EHBA (Marret et al., 1997 and Mac Mahon et al., 1998).

Reovirus is a RNA virus that belongs to reoviridae family. It is essentially an animal pathogen but its association with neonatal cholestasis is widely discussed. The virus is not associated with adult disease and its transmission to the newborn is likely to be perinatal (Sokol and Mack, 2001). Infection with reovirus serotype 3 (Reo- III) has been postulated to be associated with extra hepatic biliary atresia, and neonatal hepatitis (Coffey and Strong, 1998).

Hepatitis B virus (HBV) is a hepatotropic DNA virus from hepadnaviridae family. HBV has the greatest risk of perinatal transmission among all hepatotropic viruses (A, D, C, E and G) (Lee, 1997). Neonatal hepatitis due to HBV may occur at any time during perinatal life, but the risk is higher when acute maternal disease occurs during the first trimester of pregnancy. However, most cases of neonatal disease are acquired from mothers who are asymptomatic carriers of HBV (Snyder and Pickering, 2000).

Despite that rubella virus may be a cause of neonatal cholestasis, the presence of obvious associated problems (congenital rubella syndrome), facilitates the diagnosis and no differential diagnosis is needed from other causes of neonatal cholestasis (Stoll and Weismann, 1997).

The aim of this work is to evaluate the role of the forementioned viral infections in the pathogenesis of cholestasis in neonates presenting with cholestatic jaundice to the Neonatology Ward of Zagazig University Hospital.

## Subjects and Methods

Sixty-two neonates were included in this study (35 males and 27 females); they were divided into 2 groups:

Group I: included 44 neonates with cholestasis; 37 cases with intrahepatic cholestasis (I H), and 7 cases with extra hepatic biliary atresia (EHBA).

Group II: consisted of 18 neonates without hyperbilirubinemia used as a control group.

The exclusion criteria included neonates diagnosed as alpha-1 antitrypsin deficiency and syndromic cholestasis. Informed consent was provided by the parents of all neonates

All cases subjected to full history taking, complete physical examination, battery of investigations including, complete blood picture, liver function tests, bleeding profile, blood cultures; serum Ig M antibodies against "CMV, Reo III, HSV I&II & Rubella" and HBsAg. Abdominal ultrasonography was conducted to all cases but radionuclide choleoscintigraphy was performed for group one only. Radionuclide studies of the hepatobiliary system were performed with <sup>99m</sup>Tc-diisopropyl iminodiacetic acid (DISDA) (Sitges, 1992).

### Viral serology

Samples collection: Blood samples were obtained at the age of 28 days. Two ml of venous blood were collected aseptically and centrifuged for 10 minutes, and then sera were kept at -70°C till tested.

Serological Methods: Samples and reagents were brought to room temperature one hour before assay; all samples were subjected to the following serological tests using ELISA:

- a- IgM anti-CMV levels those were determined using commercial ELISA kits (Dia- Pro- Company)
- b- IgM anti-Rubella virus levels as determined using commercial ELISA kits (Nova Tec Immunodiagnostica).
- c- Serum IgM anti-Reovirus type 3 levels were estimated using commercial ELISA kits (Mag-Well Company).
- d- Serum IgM anti HSV I and II estimation using commercial ELISA kits (Genzyme Virotech Company).
- e- Serum HBsAg estimation using commercial ELISA kits (Linear Chemicals Company).

### Principle of the assay

The antibody searched for in the human serum forms an immune complex with the antigen coated on the test strips. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is removed by washing processes. The enzyme captured on the solid

phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of IgM antibodies present in the sample and can be detected by an ELISA reader.

#### Statistical analysis

Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Chicago). Student t-test and Chi-square ( $\chi^2$ ) test was performed and a significant level was taken at  $P < 0.05$ .

## Results

The clinical profile of 62 neonates included in the study were analyzed, hepatomegaly, dark urine and pale stool were found significantly higher in neonates with cholestasis than control group (table 1). AST, ALT,  $\gamma$ GTP, PT, and PTT were significantly higher in cholestatic group than control group (table 2).

Table 1. Comparison between cholestatic and control groups as regard sociodemographic and clinical findings.

	Cholestatic(n=44)	Control(n=18)	P - value
	No (%)	No (%)	
Gender:			
Male	25(56.8)	10(55.6)	NS
Female	19(43.2)	8(44.4)	
Delivery:			
NVD	33(75)	13(72.2)	NS
CS	11(25)	5(27.8)	
Dark urine	15(34.1)	0(0)	0.004
Pale stool	29(65.9)	4(22.2)	0.002
Hepatomegaly	26(59.1)	4(22.2)	0.008
Splenomegaly	5(11.4)	2(11.1)	NS

P > 0.05 is non significant.; NS =non significant

Table 2. Comparison between cholestatic and control groups as regard laboratory parameters.

	Cholestatic (n=44)	Control (n=18)	P - value
	Mean $\pm$ SE	Mean $\pm$ SE	
Hb	13.3 $\pm$ 0.4	13.8 $\pm$ 0.6	NS
WBC's	14.5 $\pm$ 0.8	13.6 $\pm$ 1.1	NS
ALT	295.8 $\pm$ 58.4	53.3 $\pm$ 17.2	<0.001
AST	373.2 $\pm$ 63.9	79 $\pm$ 28.4	<0.001
Alkaline phosphatase	405.9 $\pm$ 61.4	255 $\pm$ 40.0	NS
$\gamma$ GTP	573.8 $\pm$ 59.7	188.2 $\pm$ 33.25	<0.001
PT	22.4 $\pm$ 1.97	16.4 $\pm$ 1.3	0.04
PTT	60.1 $\pm$ 4.3	40.3 $\pm$ 2.1	0.002

Hb: hemoglobin

WBCs: white blood cells

ALT: Alanine transaminase

PT: prothrombine time

PTT: partial thromboplastin time

AST: Aspartate transaminase

P > 0.05 is non significant.; NS =non significant

Among the 44 cases of cholestasis, 15 cases were positive for CMV, HSVI and

ReovirusIII while in control group only one neonate was positive for HSVI (table3).

Table 3. ELISA positive cases for the studied viruses.

	Cholestatic (n=44)		Control (n=18)	
	No	(%)	No	(%)
CMV + ve	6	(13.63)	0	(0)
HSV I + ve	2	(4.54)	1	(5.55%)
HSV II + ve	0	(0)	0	(0)
Rubella + ve	0	(0)	0	(0)
Reo III + ve	7	(15.91)	0	(0)
HBsAg+ve	0	(0)	0	(0)
Total	15	(34.08)	1	(5.55)

CMV and Reovirus III IgM were significantly higher in cholestatic than control group (table 4). Among cholestatic group, there was no

significant difference between IH and EHBA as regard viral antibody titres. (table 5)

Table 4. IgM antibody titre against five of the studied viruses among cholestatic and control groups.

Virus	Antibody titre in cholestatic group (n= 44) mean±SE	Antibody titre in control group (n= 18) mean±SE	P - value
CMV	0.144±0.03	0.023±0.005	<0.001
HSV-I	0.051±0.004	0.043±0.006	NS
HSV-II	0.026±0.004	0.022±0.003	NS
Rubella	0.443±0.03	0.49±0.03	NS
Reovirus III	0.221±0.036	0.105±0.007	<0.04

P >0.05 is non significant; NS =non significant

Table 5. Comparison between neonates with (IH) and neonates with (EHBA) as regard viral antibody titre.

	EHBA(n=7)	IH (n=37)	P - value
CMV	0.194 ± 0.12	0.134 ± 0.027	NS
Reovirus III	0.341 ± 0.14	0.198 ± 0.03	NS

P >0.05 is Non Significant.; NS =non significant

## Discussion

The clinical data of the patients revealed that pale stool, dark urine and hepatomegaly were significantly associated with the cholestatic group as compared with control group; however, splenomegaly was not significantly

associated with cholestatic group. These findings were in accordance with Mac Mahon et al. (1998), who reported hepatomegaly, clay stool and dark urine in neonates suffering from cholestasis but stated that splenomegaly is a late sequelae, usually presented after the neonatal period. Liver function tests in this

study showed significantly higher levels of ALT, AST,  $\gamma$ GTP, PT and PTT in cholestatic group than in control group. However; alkaline phosphatase level, although being higher in cholestatic group versus control group, the difference was not statistically significant. This could be explained by the non-specific origin of alkaline phosphatase, which may be produced by various tissues including bone, kidney, and small intestine, thus, alkaline phosphatase as a marker of liver pathology is less reliable than other tests (Agata and Balistreri, 1999).

Among 44 cases of cholestasis, six cases were positive for CMV (13.6%), seven cases were positive for Reovirus type III (15.91%) and two cases were positive for HSV type I (4.5%). No positive cases for HSV type II, rubella or HBsAg were detected.

Prado and his Co-workers (1999) performed serological assessment for viral infections in 185 infants with prolonged cholestasis. Their results were much lower than ours regarding CMV (2.2% vs. 13.6%), Reo III (0% vs. 15.91%), slightly higher in HBV (2.4% vs. 0%) and the same for rubella and HSV 0%. These differences regarding CMV and Reo III may be due to different epidemiology of these viruses in different locations. Another factor is the late time of sampling in their study (late infancy), so IgM antibody titre may have been lowered by time. On the other hand, Oliveira and his Colleagues (2002) detected 22 positive cases of CMV IgM from 76 cholestatic neonates (29%). This study was done in Brazil, so the percentage may be higher than our study as maternal CMV infection may be higher in Brazil than Egypt.

This study showed non-significant titres against HSV I and HSV II in the cholestatic group. These results agreed with Prado et al. (1999) who stated that HSV has no role in neonatal cholestasis. On the other hand, many authors suggested a link between HSV infection and cholestasis (Sokol and Mack,

2001 and Oliveira et al., 2002); these studies were conducted in Western countries in which HSV is very common in adults (Fleming et al., 1997). In Egypt HSV; a sexually transmitted virus is not common in the general population including pregnant females.

This study revealed a non-significant difference regarding IgM anti-rubella antibodies between the two studied groups. This was in agreement with Yachha and his Co-workers (1996) who examined 60 Indian neonates with cholestasis over 3.5 years and found no significant association with rubella infection. The same result was reached in Brazil by Prado and his colleagues (1999). The overall incidence of rubella infection becomes very low since immunization becomes available. Also, awareness of physicians of the dangers of congenital rubella has resulted in performing abortion to most pregnant females catching rubella in the first trimester of pregnancy (Mac Mahon et al., 1998).

In the present study, a significantly higher level of serum anti-Reo III IgM was detected in patients group versus the control group. This result was found by many authors, e.g, Richardson and his colleagues (1994) who gave a 50% prevalence of Reo III antibodies in neonates suffering from cholestasis in Australia compared with <10% in control neonates. On the other hand, Steele and his Co-worker (1995) disputed this association after performing their study on 50 neonates. Yoon and his colleagues (1997) explained this discrepancy between studies by the presence of other factors as sex, birth weight and seasonal variation which affect viral infection in different studies while Sokol and Mack (2001) accounted for it by the different methodology for viral detection and the variable time of sample collection.

With the implementation of routine screening of blood products for HBsAg, perinatal transmission of hepatitis B from infected mothers is the possible route of

neonatal infection. As the prevalence of hepatitis B in Egypt only ranges from 2.2 %Reda et al. (2003) to 4% Schartz (1999) and not all affected mothers transmit the virus to their babies, absence of HBsAg positive neonates in the study was expected.

This study revealed non-significant differences between cholestatic neonates with EHBA (7 cases) and IH cholestasis (37 cases) regarding anti CMV IgM or anti Reo III IgM. This means that both of them may be involved in the pathogenesis of biliary atresia as well as neonatal hepatitis. A lot of studies have proved the role of CMV in both EHBA and IH cholestasis including Fischler and his Colleagues (1998) who performed their studies in Sweden infants with cholestasis. Oliveira and his Colleagues (2002) conducted a study on 76 neonates in Brazil and diagnosed CMV infection in 29.4% of neonates with IH cholestasis and 28.5% of neonates with EHBA (non significant difference). Accordingly, they stated that CMV could produce either extra hepatic or intrahepatic cholestasis; the same conclusion was reported in a recent study in Thailand by (Wongswasdi et al., 2003<sup>1</sup>).

Likewise; our study revealed that Reo-III is involved in IH cholestasis and EHBA (as was CMV), a result compatible with (Richardson et al., 1994 and Sokol and Mack 2001). The capability of viral infection to produce either neonatal hepatitis or biliary atresia can be explained by Landing hypothesis which stated that biliary atresia and neonatal hepatitis are different manifestations of a single pathological process (Landing 1974, Park et al., 1996 and Karmarkar, 2001).

Regarding the 6 studied viruses, this, study revealed significantly more positive cases of viral infection in cholestatic group (15 out of 44) compared with control group (1 out of 18) indicating a role of viral infection in neonatal cholestasis. This association was postulated by many authors (Richardson et al., 1994, Mac Mahon et al., 1998, Sokol and Mack

2001, Oliveira et al., 2002). Yoon et al. (1997) proved a significant seasonal clustering of biliary atresia, which supports the theory, that biliary atresia is caused by exposure to environmental factor (consistent with a viral cause). The sudden onset and progressive inflammation of biliary tract in neonatal hepatitis and biliary atresia strongly suggest a role of infectious agents, mainly viruses (Barton et al., 2003).

It can be conclude that CMV and Reovirus type 3 infections in the neonates are associated with the development of cholestatic disorder, not only due to IH cholestasis but also due to the production of EHBA.

## References

1. Agata ID, Balistreri WF (1999): Evaluation of Liver Disease in the Pediatric Patient. *Pediatrics in Review*; 20(11): 376-90.
2. Barton E, Yoree B, Ebert D, et al. (2003): Utilization of sialic acid as a coreceptor is required for reovirus induced biliary disease. *J Clin Invest*; 111(12): 1823-33.
3. Burchett SK (2003): Viral Infections. In: Cloherty J P, Eichenwald E C, Stark A R (eds.) *Manual of neonatal care* (5<sup>th</sup> edition): J.Lippincott Williams & Willkins.; P 255-87.
4. Coffey MC, Strong JE (1998): Reovirus therapy of tumors with activated Ras Pathway. *Science*; 282:1332-4.
5. Domiati-Saad R, Dawson DB, Margraf LR (2000): Cytomegalovirus and human herpes virus 6, but not human papillomavirus, are present in neonatal giant cell hepatitis and extra hepatic biliary atresia. *Pediatr Dev Pathol*; 3: 367-73.
6. Fischler B, Ehrnst A, Forsgren M, et al. (1998): The viral association of neonatal cholestasis in Sweden, a possible link between CMV infection and EHBA. *J Pediatr Gastroenterol Nutr*; 27(1): 57-64
7. Fischler B, Papadogiannakis N, Nemeth A (2001): Etiological factors in neonatal cholestasis. *Acta Paediatr (Norway)*; 90(1): 88-92.
8. Fleming DT, McQuillan GM, Johnson RE (1997): Herpes simplex type 2 in the United States, 1976 to 1994. *N Eng J Med*; 337: 1105-11.

9. Hicks J, Barrish J, Zhu SH (2001): Neonatal syncytial giant cell hepatitis with paramyxoviral like inclusions. *Ultrastruct Pathol*; 25(1): 65-71.
10. Karmarkar S (2001): Jaundice in early infancy. [www.pediatriconcall.com](http://www.pediatriconcall.com).
11. Landing BH (1974): Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst. *Prog Pediatr Surg*; 6: 113-39.
12. Lee WM (1997): Hepatitis B virus infection. *N Eng J Med*; 337:1733.
13. MacMahon JR, Stevenson DK, Oski FA (1998): Obstructive jaundice due to biliary atresia and neonatal hepatitis. In: Taeusch H W, Ballard R A R (eds). *Avery's Diseases of the Newborn* (7<sup>th</sup> edition) WB Saunders Company; P 1021-9.
14. MacMahon JR, Stevenson DK, Oski FA (1998): Other conjugated hyperbilirubinaemia. In: Taeusch H W, Ballard R A R (eds). *Avery's Diseases of the Newborn* (7<sup>th</sup> edition) WB Saunders Company; P 1030-2.
15. Marret S, Buffet-Janvresse C, Metayer J, Fessard C (1997): Herpes simplex hepatitis with chronic cholestasis in a newborn. *Acta Paediatr*; 82(3): 321-3.
16. Oliveira NL, Kanawaty FR, Costa SC and Hessel G (2002): Infection by cytomegalovirus in patients with neonatal cholestasis. *Arq Gastroenterol*; 39(2): 132-6.
17. Park WH, Kim S, Park K, et al. (1996): Electron microscopic study of the liver with biliary atresia and neonatal hepatitis. *J Pediatr Surg*; 31(3): 367-74.
18. Prado ET, Araujo M, Campos JV (1999): Prolonged neonatal cholestasis; prospective study. *Arq Gastroenterol*; 36(4): 185-94.
19. Reda AA, Arafa MA, Youssry AA, et al. (2003): Epidemiologic evaluation of the immunity against Hepatitis B in Alexandria, Egypt. *Eur J Epidemiol*; 18(10): 1007-11.
20. Richardson SC, Bishop RF and Smith AL (1994): Reovirus serotypes 3 infections in infants with extra hepatic biliary atresia or neonatal hepatitis. *J Gastroenterol Hepatol*; 9(3): 264-8.
21. Schartz GC (1999): Hepatitis B global infection rates. WHO, National Centre of Disease Control and Prevention. P. 105.
22. Sitges A (1992): Isotope studies in children: An update. *Seminars in Ped Surg*; 221 (1): 245-52.
23. Snyder JD, Pickering LK (2000): Viral hepatitis. In: Behrman R R, Kliegman R M, Jenson H B (eds). *Nelson Textbook of Pediatrics* (16<sup>th</sup> edition), WB Saunders Company.; P 768-76.
24. Sokol RJ, Mack C (2001): Etiopathogenesis of biliary atresia. *Semin Liver Dis*; 21(4): 517-24.
25. Steele MI, Marshall CM, Lloyd RE, et al. (1995): Reovirus III not detected by reverse transcriptase-mediated polymerase chain reaction analysis of preserved tissue from infants with cholestatic liver disease. *Hepatol*; 21(3): 697-702.
26. Stoll BJ, Weismann LE (1997): Infections in perinatology. *Clin Perinatol*; 24:1.
27. Tarr PI, Haas JE, Christie DL (1996): Biliary Atresia, Cytomegalovirus and age at referral. *Pediatr*; 97(6): 828-31.
28. Taylor GH (2003): Cytomegalovirus. *American family physician*; 67(3):519-24.
29. Wongsawasdi L, Khatiyapong V, Singhve J, et al: Infantile cholestasis syndrome at Chiang Mai University Hospital. *Chiang Mai Med Bull* 2003; 42(1): 17-23.
30. Yachha SK, Khanduri A, Kumar M, et al. (1996): Neonatal cholestasis syndrome. *Indian Pediatr*; 33(9): 729-34.
31. Yoon P, Bresee J, Olney R, et al. 1997: Epidemiology of Biliary Atresia. *Pediatr*; 99(3): 376-82.