Short Communication

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Paediatrics Section

A Study on Seroprevalence of Hepatotropic Viruses in Neonatal Cholestasis Patients at a Tertiary Care Hospital of Central India

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ABSTRACT

Introduction: Neonatal Cholestasis (NC) is defined as conjugated hyperbilirubinaemia in infancy, Diverse aetiologies and several disorders are responsible for this hepatobiliary dysfunction. Hepatotropic viral infection may have an important role in the pathogenesis of NC and related clinical outcomes.

Aim: This study was aimed to estimate the seroprevalence of the hepatotropic viruses and their possible role in neonates presenting with cholestatic jaundice.

Materials and Methods: This retrospective study included 51 infants who presented with cholestatic jaundice. Serum samples were collected and screened for the hepatotropic viruses. The presence of serological markers to Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis C (HCV), Hepatitis E (HEV), Herpes Simplex Virus (HSV) and Rubella were tested using enzyme-linked immunosorbent assays. Hepatitis B Virus (HBV) serostatus was determined by using rapid card tests.

Results: Of the 51 cases, 4 (7.6%) patients presented with biliary atresia. Seroprevalence of CMV (23.5%) was found to be more predominant followed by EBV (13.7%), HCV (5.8%), HEV (5.8%), HSV (1.9%) and Rubella (1.9%). Co-infections were found in 7 (13.7%) cases and CMV-EBV co-infection being the most common.

Conclusion: The presence of specific serological markers to hepatotropic viruses in the NC cases strongly suggests their aetiological role in this disorder. To the best of the knowledge, this is the first report documenting the seroprevalence of hepatotropic viruses in NC patients from Central India.

Keywords: Biliary atresia, Cholestatic jaundice, Congenital viral infection, Infants

INTRODUCTION

Neonatal cholestasis is characterised by conjugated hyperbilirubinemia and recognised as an important cause of chronic liver disease in infants. In India, NC constitutes 19% to 33% of all chronic liver diseases in children reporting to tertiary care hospitals [1]. Persistent cholestasis from any cause leads to liver damage and cirrhosis. Therefore, determining the specific aetiology at the earliest is critical. NC results from diminished bile flow and may be caused by either an extrahepatic (biliary atresia) or intrahepatic (nonbiliary atresia) disorders. Obstructive bile duct disorders are the most frequent and severe cause of NC. In some cases, aetiology of NC includes infections or may be part of a syndrome/disorder e.g., metabolic disorders, storage diseases, endocrine disorders, toxic or secondary disorders, immunological disorders, vascular malformations and other disorders [2,3]. These expanding groups of aetiologies of NC presented with overlapping clinical presentations.

Infectious causes of NC include both viral and bacterial aetiologies. The viral infection is one of the postulated causes of NC. Hepatotropic viruses including hepatitis viruses (A-E) and other hepatotropic viruses (adenovirus, CMV, coxsackievirus, EBV, echovirus, enterovirus, HSV, Human Immunodeficiency Virus (HIV), parvovirus, reovirus, rubella) are the main presumed infective causes of neonatal hepatitis [3]. These hepatotropic viruses may have a crucial role in the pathogenesis of NC and related clinical outcomes. Congenital or perinatal viral exposure may lead to the destruction of biliary epithelium by the hepatotropic virus itself or a secondary autoimmune reaction [4,5].

Timely identification of NC is mostly done by biochemical, imaging, histopathological and noninvasive liver stiffness evaluations. However, infectious and genetic evaluations are not routinely done. If infectious causes are suspected, blood and urine cultures should be done for bacterial identification; serology and molecular assays should be done for viral identification. These evaluations are more important in order to quickly identify the treatable infectious causes [6,7]. Serological findings of hepatotropic viruses

in NC cases and their aetiological role has been rarely documented in the country. Most of the studies have only documented the prevalence and the association of CMV in NC cases. The epidemiology of NC has been documented from Central India in very few studies [8]. Hence, the present study was aimed to investigate the possible viral agents associated with NC and to estimate the seroprevalence of hepatotropic viruses (CMV; Epstein-Barr; Hepatitis B, C & E, Herpes simplex; Rubella) in NC patients at a tertiary care hospital, Central India.

MATERIALS AND METHODS

This retrospective study was conducted in the tertiary care institute in Central India over a period of one year (January 2019 to December 2019). Ethical approval was obtained from Institutional Ethics Committee (2106768/MC/IEC/2018). About 1 mL venous blood sample was collected in the plain vial by the paediatrician and sent the serology lab for the routine investigations (TORCH panel). All the relevant clinical data were collected, including the presenting clinical features (abdominal pain, dark urine, fever, hepatomegaly, jaundice, vomiting) and the liver function test parameters. Serum was separated from each sample after centrifugation and stored in the -20°C. Infants with a provisional diagnosis of cholestatic jaundice were included for this study and their samples were retrieved from the archived storage. NC or the conjugated hyperbilirubinemia was determined by using the biochemical parameters (If the levels of serum direct/conjugated bilirubin concentration greater than 1.0 mg/dL, if the total serum bilirubin is <5.0 mg/dL or greater than 20 percent of TSB, if the TSB is >5.0 mg/dL than it will be considered as conjugated hyperbilirubinemia in a neonate) [1].

Serum samples of infants with conjugated hyperbilirubinemia were tested for the presence of viral markers. Serum was assessed for anti-CMV Immunoglobulin M (IgM); anti-rubella IgM; anti-HSV 1&2 IgM (Ratio Diagnostics, Germany), EBV (VCA) IgM (NovaTec Immundiagnostica, Germany), anti-HCV total antibodies (ErbaTransasia, India) and anti-HEV IgM (MBS New SRL, Italy). All assays were performed using commercial kits based on the enzyme-linked immunosorbent assay (1st Gen- Enzyme-Linked Immunosorbent Assay (ELISA)) as per the manufacturer's instructions. The intensity of the colour was measured using Infinite® F50 Tecan ELISA reader. The serum was analysed for Hepatitis B surface antigen (HBsAg) using rapid test kits (Angstrom Biotech, India) following the manufacturer protocols.

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel. Descriptive statistics were used to see the seroprevalence and other associated variables. Chi-square test with 95% Confidence Interval (CI) was computed to assess the associations of clinical features with the seropositivity and a $p<0.05$ was considered to be statistically significant.

RESULTS

A total of 51 infants, presented with conjugated hyperbilirubinemia were included for the study. Out of the 51 infants, 30 (58.6%) were male and 21 (41.7%) were female. The median age of presentation was 85 days (range 15-270 days). The presenting clinical features among the study population were jaundice (73%), fever (49%), hepatomegaly (49%), abdominal pain (43%), dark urine (27%) and vomiting (18%) [Table/Fig-1]. Significant associations of clinical features were not found in viral aetiology confirmed cases with that of negative cases.

Clinical features	Viral aetiology positive cases (n=19) (%)	Viral aetiology negative cases (n=32) (%)	p-value*
Abdominal pain	10 (52.6)	12 (37.5)	0.291#
Dark urine	06 (31.5)	08 (25)	0.610#
Fever	08 (42.1)	17 (53.1)	0.446#
Hepatomegaly	09 (47.3)	16 (50)	0.855#
Jaundice	14 (73.6)	23 (71.8)	0.888#
Vomiting	03 (15.7)	06 (18.7)	0.788#

[Table/Fig-1]: Clinical features of Neonatal Cholestasis (NC) cases. *Chi-square test; #p-value not significant

Of 51 cases, 4 (7.8%) were with a confirmed diagnosis of biliary atresia and 47 (92.1%) were with cholestasis due to causes intrahepatic (nonbiliary atresia) disorders. The viral aetiology was confirmed in 19 (37.2%) cases while in 32 (62.7%) cases none of the tested viruses could be detected. CMV was found in the maximum number of the cases (23.5%), followed by EBV (13.7%), HCV (5.8%), HEV (5.8%), HSV (1.9%) and Rubella (1.9%). HBsAg was negative in all the cases [Table/Fig-2]. Co-infections with more than one virus were present in 7 (13.7%) cases and the co-incidence of CMV-EBV was found to be 4 (7.8%) followed by CMV-HEV 1 (1.9%), HCV- HEV in 1 (1.9%). Co-infection of EBV-HCV-HSV was found in 1 (1.9%) extrahepatic biliary atresia case.

DISCUSSION

NC is caused by a number of factors and viral aetiology of NC is less studied. Viral infections, congenitally or perinatally acquired, may result in injury to the biliary epithelium or virus triggered immune modulated response [8,9]. Involvement of the hepatotropic viruses (adenovirus; CMV; coxsackievirus; EBV; echovirus; enterovirus; HBV; HCV; HEV; HSV; HIV; parvovirus; reovirus; rubella) may contribute the pathogenesis of NC and related clinical outcomes [2].

Viral aetiology	Neonatal cholestasis cases (n=51) (%)
Single aetiology	
CMV	7 (13.7)
EBV	2 (3.9)
HCV	1 (1.9)
HEV	1 (1.9)
Rubella	1 (1.9)
Co-infection	
CMV-EBV co-infection	4 (7.8)
CMV-HEV co-infection	1 (1.9)
HCV-HEV co-infection	1 (1.9)
EBV-HCV-HSV co-infection	1 (1.9)

[Table/Fig-2]: Prevalence of viral aetiologies of Neonatal Cholestasis (NC) cases.
CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus

Majority of the studies documented only the seroprevalence of CMV in NC patients while in the present study, the prevalence of other hepatotropic viruses were also assessed [8,10]. In present study, CMV (23.5%) was found to be more prevalent among study subjects, who were from the tertiary care hospital of Central India, Bhopal. In the literature, the seroprevalence of CMV in NC cases varies between 11% and 32% in countries like Brazil, Egypt and Sweden [8-12]. There are only few studies that documented EBV-induced cholestatic hepatitis in infants [13,14]. A study from Turkey showed 5% EBV infection while present study reports 13.7% [15]. The seroprevalence for anti-HCV-Ab and anti HEV IgM was found to be 5.8% and HBsAg was not detected. Co-infections with more than one virus were seen in 13.7% of infants and CMV-EBV was found to be more predominant (7.8%). Individuals with co-infection were presented with most the clinical features and one case was found with extrahepatic biliary atresia. To best of the knowledge, no data available from the country regarding the incidence of hepatotropic viruses in association with conjugated hyperbilirubinemia cases among the infant population. This was the first report documenting the seroprevalence of hepatotropic viruses in NC patients from the Central India.

Limitation(s)

In this study, the other probable viral aetiologies (HIV, echovirus, entervirus, adenovirus, coxsackievirus, Parvo B19 virus and HHV6-8 virus) were not studied. As this was a seroprevalance study, the molecular detection of CMV DNA was not done which is considered as a gold standard.

CONCLUSION(S)

To conclude, the presence of specific hepatotropic virus antibodies in the NC cases strongly suggests their aetiological role in this disorder. The diagnosis and clinical management of NC cases requires a combination of clinical and laboratory features and serological findings of hepatotropic viruses.

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