ORIGINAL RESEARCH

Clinical Profile of Neonatal Cholestasis in Neonatal Septicaemia

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ABSTRACT

Background: Cholestasis is a known complication of gram-negative bacterial infection, especially in infants. This syndrome is more frequent in the neonatal period and may account for as much as a third of the cases of neonatal jaundice. Objectives: to determine the prevalence of neonatal cholestasis in neonatal sepsis and to determine the prevalence of Gram negative septicemia in neonatal sepsis.

Materials and Methods: This retrospective, observational study was conducted among 267 neonates attending in neonatal ward department of pediatrics at Dr. B.R.A.M. Hospital, Raipur, Chhattisgarh.

Results: Only 47 infants out of 267 cases were having Cholestasis jaundice. Overwhelming majority of infection in our study were caused by gram negative organism Klebsiella pneumoniae (78.72%), E.coli (17.02%), Acinobacter (2.13%) and S.aureus (2.1%). Association of Cholestasis with infecting organism in subjects with neonatal sepsis was assessed using Chi square test. No significant association was found to exist between two parameters (p=0.07).

Conclusion: It was concluded that majority of infection in our study were caused by gram negative organism Klebsiella pneumonia, E.coli, Acinobacter and S.aureus.

Keywords: Neonatal Cholestasis, Neonatal Septicaemia, Gram Negative Septicemia, Jaundice.

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INTRODUCTION

Neonatal cholestasis is defined as impaired bile formation or bile flow resulting in accumulation of biliary substances (bilirubin, bile acids and cholesterol) in blood and extrahepatic tissues. This can occur anywhere between the sinusoidal membrane of the hepatocyte and the ampulla of vater. It is generally associated with a measured conjugated (direct-acting) bilirubin. Fraction of greater than 2mg/dl or more than 20% of the total bilirubin.[1]

Jaundice Is a well-known complication of sepsis or extrabacterial infection. Sepsis and bacterial infection are responsible for jaundice in newborns and early infants varies between 20% and 60%.

Sepsis is more likely to manifest with jaundice in infants and children than in adults. In this population, males have a higher incidence of jaundice.

Jaundice has been associated with infections caused by several organisms including aerobic and anaerobic gram negative and gram-positive bacteria. Gram-negative bacteria cause most of these cases. The primary site of infection is most often intraabdominal, but infection of various other sites such as urinary tract infection, pneumonia, endocarditis, and meningitis have been associated with this complication.

The pathogenesis of jaundice in systemic infections is multifactorial.The development of jaundice may occur from an aberration in the processing of bilirubin by hepatocytes or from other effects on the liver that lead to the accumulation of bilirubin in the body. Such processes include increased bilirubin load from haemolysis, hepatocellular injury, and cholestasis from the septic state and from various drugs used for the treatment of sepsis.[2]

The molecular and biochemical mechanisms by which jaundice develops in subjects with sepsis is best considered in the context of normal bilirubin metabolism.

The development of haemolysis causes an increased bilirubin load in septic individuals. Haemolysis contributes to jaundice in sepsis, it is unlikely that it is the principal mechanism because the jaundice results from conjugated hyperbilirubinemia.

Haemolysis may occur by multiple mechanisms in the setting of bacterial infection.21-These may be categorized as mechanisms of haemolysis .

- Associated with normal red cells and
- Related to underlying red cell defects.

The severe forms of many infections from gram-positive and gram-negative bacteria have been associated with haemolysis of normal red cells. Of these bacteria, Clostridium perfringens can give rise to severe, often fatal haemolysis in persons with normal red cells. Other infections that commonly cause haemolysis in normal red cells are malaria and babesiosis. Escherichia coli infection periodically may lead to haemolysis of normal red blood cells (RBCs).

Most cases of sepsis associated with cholestatic jaundice have evidence of gram-negative bacteraemia, with Escherichia coli the more common pathogen.

Pyelonephritis, peritonitis, appendicitis, diverticulitis, pneumonia, and meningitis are types of infections observed to cause jaundice.

The urinary tract is the most common site of infection associated with this syndrome, especially in the neonatal period. Liver histology shows intrahepatic cholestasis with Kupffer cell hyperplasia and little or no evidence of cellular necrosis. Aside from cholestasis, liver histology reveals an almost normal hepatic parenchyma.

The manifestations of the underlying infection usually dominate the presentation. Jaundice and cholestasis are usually reversible and subside completely after resolution of the infection.

New born infants are more prone to develop cholestasis because of immaturity of excretory function, inborn errors manifesting in early life and inherent susceptibility to various viral, septic and toxic insults. The immature liver cells are associated with peculiar kind of pathological response to different kind of noxious agents during neonatal life and infancy.

The excretory function is further compromised by the ineffective enterohepatic circulation of bile. There is gradual maturation of hepatocytes in respect to handling of bilirubin, excretion of bile, synthetic functions and enzymes system during infancy.[3]

The maturation of these functions may be equivalent to adulthood by age of 4-6 months.

Some biochemical markers of cholestasis like alkaline phosphatase and glutatnyl transpeptidase are raised during early life. Serum unconjugated bilirubin, bile concentration are normally in higher concentration in blood again suggesting that there are clearance problems in neonatal liver. Due to these reasons the neonatal hepatobiliary system is affected by various infective, metabolic and obstructive causes faster as compared to older children and adults.

This study mainly focuses on to determine the prevalence of neonatal cholestasis in neonatal sepsis and to determine the prevalence of Gram negative septicemia in neonatal sepsis.

MATERIALS & METHODS

This retrospective, observational study was conducted among 267 neonates attending in neonatal ward department of pediatrics at Dr. B.R.A.M. Hospital, Raipur, Chhattisgarh. who met inclusion criteria was taken up for study duration of the study period from September 2015 to September 2016 (1 year). Permission for the study was obtained from the College authorities prior to commencement.

Inclusion criteria: Neonates with confirmed blood culture positive sepsis from neonatal intensive care unit of Pt JNM medical college and Dr BR Ambedkar memorial hospital, Raipur.

Exclusion criteria: Neonates with IEM

- Neonates with culture negative hepatobilliary dysfunction.
- Neonates with major congenital malformation were excluded.

Baseline informatiom of mother and infant was recorded.

Clinical assessment of jaundice, liver and spleen size, color of urine and stool were looked for.

Brief maternal history was taken for fever, bleeding per vaginum, foul smelling liqour, family history of cholestatic jaundice.

Only those neonates with blood culture positive with pathogenic bacteria were included in study.

Liver function test [total and direct billirubin, SGOT,SGPT(ALT)] was done in babies with clinically evident sepsis as early as 72 hours.

Symptoms such as poor feeding, bleeding, respiratory distress were suggestive of sepsis.

I ml of venous blood collected in blood culture bottles for bacterial blood culture.

The report was available after 72 hours of incubation. Sepsis was managed as per Institute's protocol.

USG abdomen, opthalmic examination and TORCH screening was done to rule out other causes of direct hyperbillirubinemia.

Statistical analysis: Data was entered into Microsoft excel and analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows software (version 18.0; SPSS Inc, Chicago). Descriptive statistics such as mean and standard deviation (SD) for continuous variables, and frequency and percentage for categorical variables were determined.

The chi-square test and fisher's exact test (when appropriate) was used to show the associations between predictor and outcome variables. The level of significance was set at 0.05. The factors which were significant by chi-square test were selected. 95% confidence intervals were calculated for each variable.

RESULTS

A total of 267 neonates were diagnosed having confirmed culture positive sepsis and were included in the study. Incidence of cholestasis was 17.66% (n=47). There are 31 males and 16 females in total of 47 patients. 65.96% are male and 34.04% are female. Male to female ratio is 1.94:1.

In our study 57.45% cases were in the gestational age 32-36 Wks, 38.30% cases were in 36-38Wks, 4.26% cases in 38-40Wks. In our study 57.45% cases were preterm, 38.30% cases were term, 4.26% cases were post-term. In our study 59.57% cases were inborn, 40.43% cases were outborn. The mean age of subject was 35.45 ±3.42 wks and mean birth weight of 2.02±0.63 kg. The majority of subjects were LBW. Only 47 infants out of 267 cases were

having Cholestasis jaundice. Overwhelming majority of infection in our study were caused by gram negative organism Klebsiella pneumoniae (78.72%), E.coli (17.02%), Acinobacter (2.13%) and S.aureus (2.1%). Association of Cholestasis with infecting organism in subjects with neonatal sepsis was assessed using Chi square test. No significant association was found to exist between two parameters (p=0.07). Majority of infants developing culture positive septicaemia were preterm (57.45%). Liver enzyme abnormality was seen in 41 subjects (15.4%) of infant having gram negative sepsis and elevated liver enzyme, conjugated bilirubin was significantly elevated but exact prevalence was not studied. Association of Cholestasis with gender in subjects with neonatal sepsis was assessed using Chi square test. No significant association was found to exist between two parameters (p=0.09). [Table 2]

Association of Cholestasis with gender in subjects with neonatal sepsis was assessed using Chi square test. Significant association was found to exist between two parameters indicating increased risk of cholestasis in inborn subjects (p=0.02). [Table 3]

Association of Cholestasis with infecting organism in subjects with neonatal sepsis was assessed using Chi square test. No significant association was found to exist between two parameters (p=0.07). [Table 4]

Out of 267 culture positive infants 41(15.4%) had hepatobilliary dysfunction(direct billirubin>20% of total with a minimum level of 2 mg/dl or ALT>50 IU/dl). Out of which 27(65.8%) were term babies. 18(44%) were preterm babies. Inborn- 28(56%). Outborn-19 (68%).

Out of the above 41, 37(90%) had Klebsiella sepsis & 8(10%) had E.coli sepsis. [Table 5]

Table 1: Various risk factors for cholestasis in neonatal sepsis

Characteristics		**Neonatal sepsis**		**Total**	**P value**
		Cholestasis +ve	**Cholestasis -ve**		
Gender	M	37	129	160	0.09
	F	16	90	106	
Place of delivery	Outborm	19	127	146	0.02
	Inborn	28	92	120	
Prematurity	Yes	27	127	154	0.3
	No	20	82	112	
Culture report	Klebsiella	37	134	170	0.07
	E Choli	8	69	77	
	Others	2	17	19	

Table 2: Risk of Cholestasis posed by gender in subjects with neonatal sepsis

Characteristics		**Neonatal sepsis**		**P value**
		Cholestasis +ve	**Cholestasis -ve**	
Gender	M	37	129	0.09
	F	16	90	

Table 3: Risk of Cholestasis posed by place of delivery in subjects with neonatal sepsis

Characteristics		**Neonatal sepsis**		**P value**
		Cholestasis +ve	**Cholestasis –ve**	
Place of delivery	Outborn	19	127	0.02
	Inborn	28	92	

Table 4: Risk of Cholestasis posed by infecting organism in subjects with neonatal sepsis

Characteristics		Neonatal sepsis		P value
		Cholestasis +ve	Cholestasis –ve	
Culture report	Klebsiella	37	134	0.07
	E Choli	8	69	
	Others	2	17	

Table 5: Prevalence of Hepatobilliary Dysfunction (n=267)

Abnormality	Total	%
No abnormality	226	84.6%
Hepatobilliary dysfunction(direct billirubin>20% of total or alt>50iu/l	41	15.4%

DISCUSSION

Most neonatal with sepsis in our study have low birth weight which is less than 2500 grams as many as 22 subjects (46.8%), with peak birth weight group between 1500-2500 grams. According to study by Shamir et al, birth weight of less than 2500 grams group are the highest population with gram negative sepsis.[4]

Other finding in this study that is in accordance with study by Shamir et al is that gestational age of less than 38 weeks are the most frequent group with gram negative sepsis. Pre-term neonates (gestational age < 38 weeks) are immature, their immune system are inadequate therefore they are susceptible to infection.[4]

In this study we found the incidence of neonatal cholestasis sepsis is 57.45%, lower compare to previous study.[5]

A retrospective study showed occurrence of cholestasis in 54 sepsis neonates at NICU and Neonatology ward Cipto Mangunkusumo National General Hospital with percentage of 74.5% proven sepsis.[4]

The difference with our study, we use larger sample size and included both proven and clinical sepsis. In our study microbial etiology of neonatal sepsis is gram negative bacteria with the most frequently found Klebsiella species(78.7%) followed by E. coli (17%), Staph areus (2.1%) and Acetobacter(2.1%). Our result is similar with reports from several refferrence that stated most neonatal sepsis are caused by gram negative bacteria. Nevertheless in our study, Fisher exact test failed to reveal significant correlation between gram negative bacteria and cholestasis occurrence.

We read the study of Khalil et al about hepatobiliary dysfunction in neonatal septicaemia with a high interest.[5] They reported cholestatic jaundice with a rate of 42.5% in Gram-negative septicaemia. They retrospectively reviewed our 5-year (2006–2011) data of neonates with Gram-negative septicaemia. Seventy of 92 infants with Gram-negative confirmed sepsis were included in the study. The remaining were excluded because of comorbitidities associated with cholestasis. The responsible pathogens were determined as Escherichia coli (n=22), Klebsiella pneumoniae (n=18), Klebsiella oxytoca (n=7), Pseudomonas aeruginosa, (n=8), Serratia marcescens (n=4), and Acinetobacter baumannii (n=11).

Similar microbial etiology result was found in previous study. They found the most frequent microbial etiology of neonatal cholestasis sepsis is Acinetobacter calcoaceticus with percentage of 58.6%. Data analysis from our study found no significant correlation between Acinetobacter calcoaceticus with the occurrence of cholestasis, p=0.975.[4]

Tiker et al report Escherichia coli is still the number one gram negative bacteria that caused neonatal cholestasis sepsis at neonatology ward (46.6.%), followed by Klebsiella pneumoniae and Pseudomonas aeruginosa. Positive gram bacteria as etiology from our study is Staphylococcus aureus (20.0%), and coagulase-negative Staphylococcus spp (13.0%).[6]

In our study, retrospective observational study, conducted in 47 neonates with blood culture–confirmed sepsis, documented that hepatobiliary dysfunction is extremely commonly seen early in the course of neonatal septicaemia. Although cholestatic jaundice (42.5%) was more common than raised ALT (37.3%), almost onefourth of the babies had both abnormalities. As an overwhelming majority (96.7%) of infections in our study were caused by Gram negative organisms, especially Klebsiella, the results are reflective of the hepatobiliary abnormalities in Gram-negative sepsis only and may not be true for Gram-positive organisms. Earlier studies from our institute and from other regions in developing countries also report Klebsiella to be the most common organism responsible for neonatal sepsis. This may be explained by higher incidence of surface colonization with this bacteria and higher virulence of Klebsiella strains in our population.[7]

Shamir et al conducted a study in premature babies with Gram-negative septicaemia (n=54) and Gram-positive septicaemia (n=31). Liver enzyme abnormality was seen in 46.3% of neonates with Gram-negative sepsis and only 12.9% of Gram-positive sepsis. In addition to elevated liver enzymes, conjugated bilirubin was significantly elevated, but the exact prevalence was not studied. These infants had significantly elevated conjugated bilirubin without increased alkaline phosphatase. Our study documented that these abnormalities are not restricted to premature infants because two-thirds of our study infants were born after completing 37 weeks.[4]

CONCLUSION

Overwhelming majority of infection in our study were caused by gram negative organism Klebsiella pneumoniae (78.72%), E.coli (17.02%), Acinobacter (2.13%) and S.aureus (2.1%). Majority of infants developing culture positive septicaemia were preterm. Liver enzyme abnormality was seen in 41 subjects (15.4%) of infant having gram negative sepsis and elevated liver enzyme, conjugated bilirubin was significantly elevated but exact prevalence was not studied.

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REFERENCES

1. Conjugated Hyperbilirubinemia in Children David Brumbaugh and Cara Mack Pediatricin Review2012;33;291
2. Rooney JC, Hill DJ, Danks DM. Jaundice associated with bacterial infection in the newborn. Am J Dis Child 1971; 122: 39¬ 41. Indian Journal of Pediatrics, May, 2006; 73: 411.
3. McKiernan PJ. The infant with prolonged jaundice: Investigation and management.Curr Paediatrics. 2001;11 (2):83-9.
4. Shamir R, Metzger AM, Bujanover Y, Ashkenazi S, Dinari G, Sirota L. Liver enzyme abnormalities in gram-negative bacteremia of premature infants. Pediatr Infect Dis J 2000
5. Khalil S, Shah D, Faridi MM, Kumar A, Mishra K. Prevalence and outcome of hepatobiliary dysfunction in neonatal septicaemia. Clin Perinatol 2002; 29: 159-180
6. Tiker, Shamir R, Maayan-Metzger A, Bujanover Y. Liver enzyme nutrition: abnormalities in gram-negative bacteremia of premature J Pediatr Gastroenterol Nutr 1989; 8: 297-303
7. Emerick KM, Whitington PF. Molecular basis of neonatal cholestasis. Pediatr Clin North Am 2002;49:221-35.