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Original Research Article

Biochemical Evaluation of Serum Bilirubin Fractions and Liver Function Tests in Sepsis Neonates with Hyperbilirubinemia

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Abstract

Background: Neonatal sepsis remains one of the leading causes of morbidity and mortality both among term and preterm infants. Sepsis and meningitis are responsible for most of these deaths. According to WHO estimates, there are about 5 million neonatal deaths a year. Jaundice and hepatic dysfunction frequently accompany a variety of bacterial infections. This study was aimed to evaluate bilirubin fractions and liver function tests in septic and non septic neonates with hyperbilirubinemia.

Materials & Methods: A total of 41 neonates, their age ranged from (1-28 days), mean age sepsis cases 4.29±5.34 and mean age in non sepsis 2.27±4.44. The patients admitted to neonatology unit for the management of hyperbilirubinemia were included in this study. Out of 41, 20 babies having sepsis (17 were males & 3 were females) and 21 (15 were males & 6 were females) were non sepsis. All study subjects were studied for the serum bilirubin fractions and other liver function tests by using vitros dry chemistry analyzer.

Results: In the present study, delta bilirubin (0.955 ±0.546) and conjugated bilirubin (1.17±2.10) levels are significantly increased in sepsis cases when compared to non sepsis controls.

Conclusion: In conclusion, conjugated bilirubin and delta bilirubin were significantly increased in neonates suffering from sepsis with hyperbilirubinemia. By studying individual fractions of bilirubin, especially unconjugated bilirubin, conjugated bilirubin and delta bilirubin (not as direct and indirect bilirubin) will help in early diagnosis of sepsis and thus may help in better management of the sepsis neonates.

Keywords: Delta bilirubin, Hyperbilirubinemia, Jaundice, Sepsis.

1. Introduction

Neonatal sepsis remains one of the leading causes of morbidity and mortality both among term and preterm infants [1,2]. Neonatal infections are a major cause of death worldwide. It is estimated that approximately 4 million deaths occur annually in developing countries in the neonatal period, attributable mostly to infection, birth asphyxia, and consequences of premature birth and low birth weight. The incidence of neonatal sepsis varies from 1-4 / 1000 live birth in developed countries, to 10-50/1000 live birth in developing countries [3]. Sepsis and meningitis are responsible for most of these deaths. According to WHO estimates, there are about 5 million neonatal deaths a year.

It is responsible for about 30-50% of the total neonatal deaths in developing countries. Sepsis can evolve to multiple organ dysfunction syndromes (MODS), whose severity accounts for a high mortality rates. During sepsis, liver dysfunction is one of the MODS components and usually is associated with a poor prognosis [4]. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related cause.

The signs and symptoms of neonatal sepsis are non specific. These include fever or hypothermia, respiratory distress including cyanosis and apnoea, feeding difficulties, lethargy or irritability, hypotonea, seizures, bulging fontanel, poor perfusion, bleeding problems, abdominal distension, hepatomegaly, gauiac-positive stools, and unexplained jaundice [1]. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections [2].

Jaundice and hepatic dysfunction frequently accompany a variety of bacterial infections. The relationship between sepsis and jaundice, particularly in a pediatric population was reported early as 1837. Jaundice is a well-

known complication of sepsis or extrabacterial infection. The jaundice of sepsis is usually cholestatis and can occur within a few days of the onset of bacteremia and may even appear before other clinical features of the underlying infection become apparent [5]. Sepsis and bacterial infection are responsible for up to 20% of cases of jaundice in patients of all ages in a community hospital setting.

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 hemolysis, hepatocellular injury and cholestasis from the septic state and from various drugs used for the treatment of sepsis [6].

The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby [7]. No single laboratory test has been found to have enough specificity and sensitivity and therefore laboratory confirmation must be used in conjunction with risk factors and clinical signs. These tests include culture of blood, urine and cerebrospinal fluid, leukocyte profile, platelet count, acute phase reactants (ESR, C-reactive protein), latex agglutination tests, or counter immune electrophoreses and Polymerase Chain Reaction (PCR) [8,9]. C-reactive protein (CRP) synthesis increases within (4-6) hrs, doubling every 8 hrs thereafter, and peaking at 36-50 hrs after the onset of inflammation. This study was aimed to evaluate bilirubin fractions and liver function tests in septic and non septic neonates with hyperbilirubinemia.

2. Materials & methods

This is a prospective study, done at PES Institute of Medical Sciences & Research, Kuppam, Andhrapradesh. A special questionnaire was designed for the purpose of the study. A total of 41 neonates, their age ranged from (1-28 days), mean age sepsis cases 4.29±5.34 and mean age in non sepsis 2.27±4.44. The patients admitted to neonatology unit for the management of hyperbilirubinemia were included in this study. Out of 41, 20 babies having sepsis (17 were males & 3 were females) and 21 (15 were males & 6 were females) were non sepsis. Newborn babies with hyperbilirubinemia, due to congenital, metabolic, other liver diseases and phototheraphy cases were excluded from the study. Institutional ethics committee clearance was obtained prior to the study. All study subjects were studied for the serum bilirubin fractions and other liver function tests by using vitros dry chemistry analyzer. Under aseptic conditions, 2 ml blood sample was collected & allowed to clot for 20 mints, centrifuged at 3000rpm. The separated serum sample was used for the estimation of total bilirubin, BuBc, aspartate transaminase, alanine transaminase, alkaline phosphatase, total proteins and albumin. Delta bilirubin was calculated by using the formula, Delta bilirubin = Total bilirubin – Unconjugated bilirubin.

2.1 Statistical analysis

Statistical analysis was done by using SPSS package, data were expressed as mean±SD. P-value <0.05 was considered as statistically significant.

3. Results & Analysis

A total of 41 neonates, their age ranged from (1-28 days), admitted to neonatology unit for the management of hyperbilirubinemia were included in this study. Out of 41, 20 babies having sepsis (17 were males & 3 were females) and 21 (15 were males & 6 were females) were non sepsis.

In the present study, delta bilirubin (0.955 ±0.546) and conjugated bilirubin (1.17±2.10) levels are significantly increased in sepsis cases when compared to non sepsis controls. Total bilirubin, unconjugated bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, total proteins and albumin levels are not shown significant difference, as illustrated in Table 1.

Table 1: Comparison of liver function test parameters between sepsis and non sepsis patients

Parameter	Sepsis Cases (n=20) Mean±SD	Non-sepsis controls (n=21) Mean±SD	P-Value
Total bilirubin	11.85±6.15	11.08±3.82	0.640
Bu	10.03±5.81	10.47±3.57	0.783
Bc	1.17±2.10	0.026±0.014	**0.029***
Delta bilirubin	0.955 ±0.546	0.602±0.532	**0.046***
Aspartate transaminase (AST)	69.23±33.88	67.18±30.25	0.854
Alanine transaminase (ALT)	82.76±127.85	26.77±12.25	0.141
Alkaline phosphatase (ALKP)	181±43.15	159.31±46.01	0.178
Total protein	5.72±0.925	5.95±0.790	0.430
Albumin	3.08±0.665	3.34±0.477	0.219
Globulin	2.65±0.535	2.57±0.413	0.652

* P value statistically significant at <0.05

4. Discussion

Neonatal sepsis may have subtle, diverse and nonspecific symptoms and signs, moreover, a delay in the diagnosis and commencement of treatment result in a high morbidity and mortality rates [3]. Earlier studies have shown that there is no clinical significance of measuring bilirubin protein conjugates, i.e. delta bilirubin.

Measurement of bilirubin protein conjugates (delta bilirubin) has been investigated in children. Because most newborns have unconjugated hyperbilirubinemia, the clinical need for delta bilirubin measurements in the newborn is non-existent. Thus measurement of delta bilirubin is not recommended for neonates or adults because of no known diagnostic value [10].

Jaundice and hepatic dysfunction frequently accompany a variety of bacterial infections. Jaundice may result either directly from bacterial products causing liver injury or as a consequence of host's response to infection. Frequently both factors contribute to the development of jaundice [6].

Mean age of sepsis with hyperbilirubinemia was 4.29±5.34 days. Hence these considered as late onset of sepsis and the mean age of non sepsis with hyperbilirubinemia was 2.27±4.44, which were mainly diagnosed as physiological jaundice with the rise in unconjugated bilirubin. The hyperbilirubinemia with sepsis and non sepsis, the distribution was more in male babies compared to female babies. i.e.84.2% were males in sepsis group and 68.2% were males in non sepsis group, which indicates, male babies are more prone for developing hyperbilirubinemia either due to inefficient or immature liver function and also due to early liver damage during sepsis.

Most of the babies in the sepsis group showed grossly elevated total bilirubin and also conjugated bilirubin. When the different bilirubin fractions were compared in cases & controls, there was significant difference in conjugated bilirubin and delta bilirubin concentration, with P values 0.029 and 0.046 respectively. But there was no significant difference in other parameters of liver function tests among the cases & controls.

Various mechanisms can lead to hyperbilirubinemia during systemic infections. The development of hemolysis causes an increased bilirubin load in septic individuals. In early studies, hemolysis was believed to be principal mechanism of jaundice in sepsis [11]. The various causes for hemolysis could be severe forms of many infection from gram positive and gram negative bacteria, especially Clostridium Perfringens [12,13]. Multiple drugs also cause hemolysis like penicillin, anti malaria medications, sulfa medications or acetaminophen [14,15].Using light microscopy. Tungswell et al found excess iron containing pigment in liver of patients with pneumonia and noted ferritin containing lysosomes in kuffer cells [16]. This was believed to be compatible with hemolysis and secondary iron overload. Although hemolysis contributes to jaundice in sepsis, it is unlikely that it is principal mechanism because the jaundice results from conjugated hyperbilirubinemia [17-19].

In our study, there is a significant raise in conjugated bilirubin among the babies suffering from hyperbilirubinemia with sepsis. And significant raise in delta bilirubin is an evident to show that the cause for hyperbilirubinemia is mainly cholestasis. In our study, we have noticed that most of the sepsis cases with hyperbilirubinemia had increased conjugated bilirubin and delta bilirubin.

5. Conclusion

In conclusion, conjugated bilirubin and delta bilirubin were significantly increased in neonates suffering from sepsis with hyperbilirubinemia. By studying individual fractions of bilirubin, especially unconjugated bilirubin, conjugated bilirubin and delta bilirubin (not as direct and indirect bilirubin) will help in early diagnosis of sepsis and thus may help in better management of the sepsis neonates. Further studies are required to establish the reference ranges for the bilirubin fractions, as there is still lot of confusion and laboratories often use the terms indirect bilirubin and direct bilirubin interchangeably with unconjugated and conjugated bilirubin respectively, but this not correct for neonates. Limitations of this study include, cross-sectional study, samples were collected only one time, prognosis was not studied.

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