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The Liver in Sepsis in Early Childhood: An Autopsy Study.

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ABSTRACT Sepsis, a leading cause of death in children between 1-4 years of age, occurs in response to infection. It commonly presents with hepatic dysfunction as seen clinically, biochemically and morphologically. We aimed to study the morphological changes in the liver in young children who died of sepsis. We also aimed to evaluate common presentations of sepsis during early childhood. The total number of autopsy cases in the 0-4 year's age group were 314, of which 185 cases were of sepsis (59%). Sinusoidal congestion was the most common microscopic feature, seen in 84.86% cases. Pneumonia was the most common primary focus for sepsis.

KEYWORDS : sepsis, neonate, infant, liver pathology

Introduction

Sepsis is the 7th leading cause of death in children between 1-4 years of age.[1] It is an inflammatory cascade that is initiated by the host in response to infection resulting from a suspected or proven infection.[2] Hepatic dysfunction is a common manifestation during sepsis, ranging from a mild elevation of serum bilirubin and/or liver enzymes to severe hepatic failure.[3]The pathophysiology of liver injury is multifactorial and involves a broad spectrum of inflammatory mediators in response to bacterial lipopoly saccharides.[3,4]

Hepatomegaly and jaundice may be associated with sepsis. Infection may reach the liver via portal vein, hepatic artery or biliary tree. Microscopic features include canalicular cholestasis, inspissated bile in the cholangioles, dilated ductules, cholangitis, hepatocellular apoptosis, fatty change, perivenular ischaemic necrosis, portal and lobular inflammation and parenchymal microabscesses.

In jaundiced septic patients, three histological patterns have been described in literature:[5,6,7]

- Canalicular cholestasis, usually most severe in zone 3,
- Ductular cholestasis with inflammation and
- Non-bacterial cholangitis associated with the toxic shock syndrome.[8]

Intrahepatic cholestasis in septicemia could be attributed to many factors such as circulating endotoxin causing functional disorders in bile secretion, disturbances in bile canalicular contraction and ischemia.[9,10,11]

Aims and Objectives

- To study the morphological changes in the liver in young children who have died of sepsis.
- To evaluate common presentations of sepsis during early childhood.

Materials and Methods

The study was done over a period of 6 years. All liver tissue and necropsy liver biopsies of children 0-4 years were selected. Approval for the study was taken from the ethics committee of the parent institution.

Inclusion criteria

Liver tissues of patients who had died of sepsis and undergone autopsy. The diagnosis of sepsis was made on the basis of clinical criteria or on the basis of positive culture reports. The clinical criteria used for diagnosis was children with following signs and symptoms suggestive of inflammation plus infection:

- o hyper- or hypothermia (rectal temperature >38.5 or < 35°C),
- o tachycardia (may be absent in hypothermic patients),
- o and at least one of the following indications of altered organ function: Altered mental status, hypoxemia, increased serum lactate level or bounding pulses[2].

Liver tissues of children in the 0-4 years age group that were diagnosed as any pathology other than sepsis, like storage disorder, leukaemia, cases of accident and death, and underwent autopsy were used as age matched control.

Exclusion criteria

Liver biopsies and necropsy biopsies of children that were too tiny and did not show even two portal areas were excluded.

Data was recorded in a case record form including clinical profile and results of laboratory and imaging studies from the records of cases in the autopsy and surgical histopathology sections (in cases where necropsy liver biopsy was done). Fixation of tissues was done with 10% formalin. Sections were taken and stained with hematoxylin and eosin dye in all cases. Special stains were used as and when required. A detailed gross and histopathological examination was carried out. The morphology of liver was studied in detail and compared with age matched controls.

Results

The total number of cases was 314, of which 185 cases were of sepsis as per criteria cited earlier. Cases of sepsis accounted for approximately 59% of the total autopsy cases in children aged 0-4 years. Most of the cases were aged 0-7 days (Figure 1). Male: Female ratio was 1.37:1 (107 males and 78 females). In 77 cases (41.6%) focus of infection was bronchopneumonia, followed by aspiration pneumonia 22 %(40 cases) and entero-colitis 7 %(13 cases) (Figure 2). The cases of sepsis were divided into 3 age groups for evaluation of laboratory parameters (neonates, infants post neonatal period, and >1-4 years. There was no significant difference between the 3 age groups.

γ-glutamyl transferase an effective marker for cell necrosis could not be studied as it is not done in our institute.

Small for gestational age was the most common contributory factor (57%) for sepsis in the study age group. The most common clinical manifestation was alteration of body temperature (71.89% cases) followed by pallor (63.24% cases). Haemoglobin was reduced in

87.62% cases. Total leucocyte count was raised in 69.14% cases and was below normal in 12.02% cases. Platelet count was reduced in 55.59% cases. Bilirubin was raised in 42.44% cases. AST was raised in 49.25% cases. ALT was raised in 47.65% cases. ALP was raised in 87.36% cases. Culture was positive in 66.63% cases. Blood was the most common specimen used for culture (92% cases). The commonest organism was Streptococcus pneumonia (30.27% cases), followed by Escherichia coli (7.57% cases) and Pseudomonas sp. (7.57%). Sinusoidal congestion was the most common microscopic feature among all age groups (seen in 84.86% cases).The other microscopic features were inflammation (periportal-69.27%, intalobular79.7%) cholestasis(hepatocellular58.47%, canalicular56.7%, ductular-22.2%), and fatty change(macrovesicular-53.04%, microvesicular-10.47%), necrosis(confluent-13.41%, zonal-19.53%, submassive-6.39%) and cholangiolitis (23.15%).

Discussion

The morphology of liver was studied in detail and compared with age matched controls. The graph of the age versus the number of cases of sepsis (figure 1) shows maximum number of cases in the newborns with a downward slope up to 3 months of age. There is a small peak at the age of 4 months to 1 year, and thereafter a fall followed by a plateau up to 4 years. Here we would like to stress the fact that the graph for the total number of cases of autopsy in the study age group also follows a similar slope and the two lines are almost parallel. Hence, we suggest that the apparent high incidence in newborns is only because of a high incidence of autopsies performed in this age group, and does not prove greater vulnerability of this age group. The higher incidence of autopsies in this age group can be explained by the fact that, there is always an increased need to find out the cause of unexplainable death especially when the child was good weight or was full-term.

The male to female ratio was 1.37:1, indicating females are slightly more protected against sepsis and mortality due to it. In a previous study, McGowan et al found a higher incidence of sepsis in men than women admitted to Boston City Hospital between 1935 and 1972[12]. Large epidemiologic studies on sepsis have found that the incidence of sepsis is 20 to 28% higher in men than women[13, 14, and 15]. Studies report that sex-based disparities in sepsis incidence exist in the neonatal and pediatric sepsis population despite insignificant hormonal differences.[16]There is evidence of a higher incidence of sepsis in men.[17]Several studies including that by Scotland RS (2011)[18] have proved that females have better innate immunity and tolerate sepsis better with lower rates of death in them.

The most common clinical manifestation of sepsis over all age groups in the present study was alteration of body temperature (71.89% cases) followed by pallor (63.24% cases) and breathlessness (52.43%cases). However, pallor (72.22% cases) was commoner than alteration of body temperature (62.04% cases) in neonates. In literature fever is reported to be the most common presenting symptom of children with sepsis.[19]

Small for gestational age (both preterm and low birth weight) was the most common contributory factor for sepsis noted in 57 cases (30.81%) followed by an unimmunized / incompletely immunized status which was noted in 42 cases (22.70%). A study by Kaufman D[20] suggests that, twenty percent of very-low-birth-weight (<1500 g) preterm infants experience a serious systemic infection, and despite advances in neonatal intensive care and antimicrobials, mortality is as much as three fold higher for these infants who develop sepsis than their counterparts without sepsis during their hospitalization.

The important factors which were associated with death in the neonates with sepsis were small for gestational age, prematurity and birth asphyxia. Literature suggests that premature infants have an increased susceptibility to sepsis and have subtle nonspecific initial presentations.[21]

The septic focus was found in all except 8 of our cases (4.32%), all of which were biopsy cases. The most common septic focus was bronchopneumonia in the lung, seen in 77 cases (41%) (Figure 2). This was followed by aspiration pneumonia in 40 cases (22%) and enterocolitis seen in 13 cases (7%). In some cases there was more than one focus. In these cases the more severely affected organ was considered to be the primarily affected organ. Koskinas et al (2008) in a similar study in adults reported respiratory infections as the most common focus (53.33% cases)[4]. In our study, culture was performed ante-mortem in 33 cases and post-mortem in 152 cases. 68.65% patients of sepsis had positive cultures with Streptococcus pneumonia being the most commonly isolated organism (30.27%). Koskinas et al (2008)[4] reported *Klebsiella pneumoniae* as the most common pathogen isolated (46.67%). Blood was the most commonly used culture material (92% cases). The results of post mortem cultures however needs to be interpreted keeping in mind the drawbacks of post mortem cultures, contamination and false-positives being the important issues. Hove M et al (1998)[22]reported a false positive rate (i.e. clinically irrelevant positivity rate) of 34% when blood was collected from the right atrium after heat searing. This is the method that was used for blood collection by us. Morris et al (2005)[23] reported that a pure growth of a known pathogen has >50% likelihood of being found in association with genuine infection. They conclude that a pure growth of a pathogen in blood or CSF should be regarded as a possible contributing factor to death at all ages.

Gross evaluation of the livers of autopsy cases of sepsis was done. The capsular surface was mostly smooth as seen in 143 cases (77.3%) and the cut surface was most commonly congested as seen in 118 cases (69.82%).The microscopic features were evaluated under 3 subgroups dividing the total septic children into neonates, infants and early childhood. This was done to detect any morphological differences in the various age groups. However on evaluation there was no significant difference in the microscopic features of the 3 groups. The most common overall microscopic finding was sinusoidal congestion seen in 84.86% cases (Fig. 3). This finding was observed and interpreted with extreme caution as mild sinusoidal congestion is a well-known post mortem finding. Controls were used to compare the sinusoidal dilatation and congestion due to post-mortem artefact and as a part of sepsis. The cases of autopsy with a diagnosis other than sepsis were used as 'within study' controls for the purpose. The cases of ante mortem biopsy where the diagnosis was sepsis also showed significant sinusoidal congestion further supporting our finding. In a study by Ceydeli A et al(2003)[24], inflammatory mediators like TNF-alpha, IL-1beta, and IL-6 correlated with liver histology of sinusoidal congestion especially centering on the central vein. In another study by Kakar S et al(2004)[25] the causes of sinusoidal dilatation and congestion in liver biopsy were studied and was found to occur in the setting of systemic inflammatory diseases.[146] The consistent features among all age groups (table 1) were lobular inflammation and portal inflammation(Fig.7), followed by cholestasis(Fig.8) and macrovesicular steatosis(Fig.4). Hepatocellular parenchymal, microabscesses (Fig.5, 6) and cholangitis followed in the list. Gradation of steatosis has been widely studied in literature. In our study mild, moderate and severe steatosis was seen in 23.78%, 37.3% and 15.14% cases respectively. These findings correlated well with literature.[4] The clinical features, laboratory results and morphological features did not show any significant variation among neonates, infants and children in early childhood.

Conclusion:

1. The most consistent microscopic finding in the liver in cases of sepsis was sinusoidal congestion (84.86%). Other microscopic findings were as described in literature.
2. Pneumonia was the most common primary focus for sepsis and Streptococcus pneumonia was the most common causative organism.
3. There was no significant difference between the morphological findings in the liver between neonates, infants and >1 to 4 years age groups in cases of sepsis.
4. The male to female ratio in cases of sepsis was 1.37:1, indicating

females were slightly more protected against sepsis and mortality due to it.

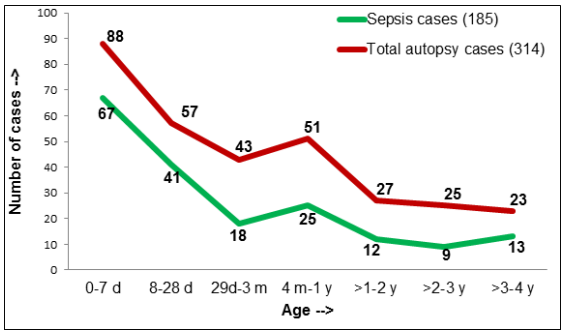

Figure 1: Line diagram showing relationship of total autopsy cases and cases of sepsis. The two curves run almost parallel to one another.

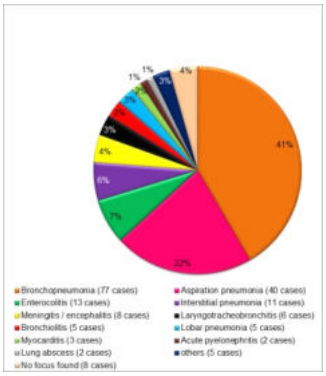

Figure 2: Pie diagram showing the primary focus in cases of sepsis (n=185).

'Others' included one case each of infected meningomyelocele, bone marrow suppression, multiple skin abscesses, vestibular cellulitis and meningococcemia.

Table 1: Microscopic features of the liver in cases of sepsis:

Microscopic feature			0 – 28 days (n=108)		29 days – 1 year (n=43)		>1 – 4 years(n=34)		Total (n=185)	
			No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
Cholestasis		Hepatocellular	70	64.81	21	48.84	21	61.76	112	58.47
		Canalicular	75	69.44	18	41.86	20	58.82	113	56.71
		Ductular	29	24.07	07	16.28	09	26.47	45	22.27
Steatosis	type	Microvesicular	15	13.89	05	11.63	02	5.88	22	10.47
		Macrovesicular	45	41.67	29	67.44	17	50	91	53.04
		Mixed	19	17.59	05	11.63	04	11.76	28	13.66
	degree	Mild	21	19.44	10	23.26	13	38.23	44	23.78
		Moderate	41	37.96	22	51.16	06	17.65	69	37.3
		Severe	17	15.74	07	16.28	04	11.76	28	15.14
Necrosis		Focal	42	38.89	10	23.26	07	20.59	59	
		Confluent	15	13.89	05	11.63	05	14.71	25	13.41
		Zone-3 ischaemic	31	28.7	04	9.3	07	20.59	42	19.53
		Submassive / massive	10	9.26	03	6.97	01	2.94	14	6.39
Inflammation		Periportal	69	63.89	29	67.44	26	76.47	124	69.27
		Lobular	76	70.37	32	74.42	29	85.3	137	79.7
		Microabscess	28	25.93	11	25.58	08	23.53	47	25.01
Cholangiolitis		27	25	09	20.93	08	23.53	44	23.15	
Sinusoidal congestion		88	81.48	40	93.02	29	85.3	157	84.86	

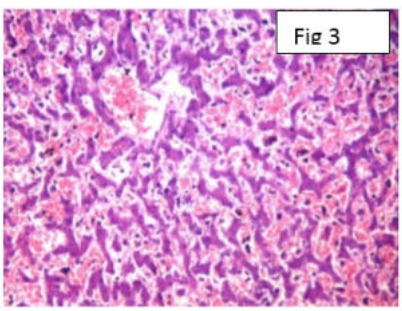

Figure 3: Sinusoidal dilatation and congestion (H&E x 100X)

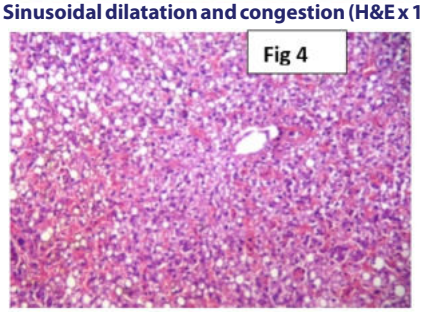

Figure 4: Macrovesicular steatosis (H&E x 100X)

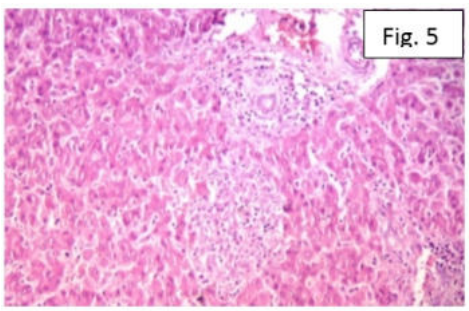

Figure 5: Focal parenchymal necrosis (H&Ex100X)

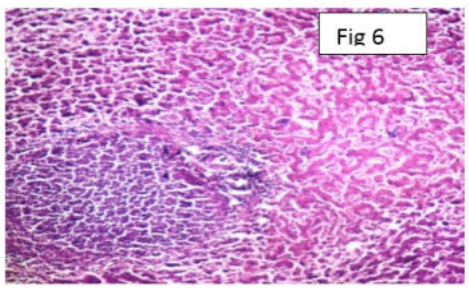

Figure 6: Parenchymal micro abscess (H&Ex100X)

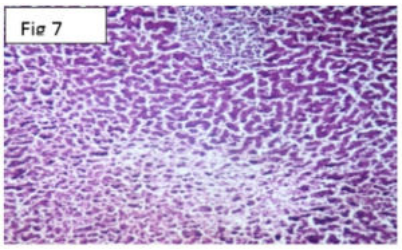

Figure 7: Necrosis, periportal inflammation (H&E x 100X)

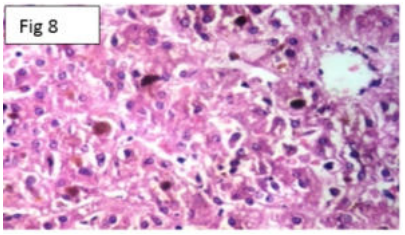

Figure 8: Intrahepatic, canalicular and ductular cholestasis (H&E x 400X)

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