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Bacteriological, Cytological and Biochemical Profile of Ascitic Fluid in Children with Chronic Liver Disease

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ABSTRACT

Introduction: *Ascitic fluid bacterial infection is a fatal condition in children, especially with chronic liver disease (CLD). Culture negative neutrocytic ascites is the commonest type of infection in children with CLD.* ***Objective:*** *To observe bacteriological, cytological and biochemical profile of ascitic fluid in Children with CLD.* ***Methods:*** *Thirty five consecutive children with clinical features suggestive of CLD, aged between 2-14 years of both sexes with clinically detected ascites and admitted at the Department of Paediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU) from January 2013 to June 2013 were enrolled in this cross-sectional study. Children who had features of ascitic fluid infection like fever, abdominal pain with tenderness were categorized as symptomatic and children who had no features of ascitic fluid infection were categorized as asymptomatic children. Demographic, clinical, haematological, biochemical and ascitic fluid study were done for both symptomatic and asymptomatic children. Variants of ascitic fluid bacterial infection was identified by ascitic fluid polymorphonuclear leucocyte count and culture report.* ***Results:*** *Among 35 children, majority 17 (48.6%) were between 6-10 years of age group with male predominance. About 12 (34.3%) were symptomatic and among the symptomatic children 7 (58.4%) were infected and 5 (41.6%) were non-infected, 16 (45.7%) had culture negative neutrocytic ascites (CNNA) variant of ascitic fluid bacterial infection as evident by ascitic fluid polymorphonuclear leucocyte count of ≥ 250/mm^3 and negative culture report. Mean ascitic fluid polymorphonuclear neutrophil count was 515±177.82/HPF among infected children and 85.47±70.60/HPF among non-infected children, which is statistically significant (p-0.001).* ***Conclusion:*** *Culture negative neutrocytic ascites (CNNA) variety of ascitic fluid infection was the only variety in this study.*

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INTRODUCTION

Chronic liver disease (CLD) is not uncommon among paediatric population in Bangladesh. It was found that 4% of hospitalized children in the Department of General Paediatrics and Paediatric Gastroenterology and Nutrition of Bangabandhu Sheikh Mujib Medical University (BSMMU) were due to liver disease and among them 40% had CLD.[1] Infections in children with CLD are an important cause of morbidity and mortality. Mechanism of increased susceptibility to infection is unclear.[2] Common bacterial infections in children with CLD are spontaneous bacterial peritonitis (SBP), urinary tract infection (UTI), community acquired pneumonia, dermatologic infections and bacteremia.[3] For the purpose of diagnosis and classification of ascitic fluid infection, culture of the ascitic fluid is essential.[4] Ascitic fluid bacteriological culture is negative in approximately 40 % of adult patients with clinical manifestation suggestive of spontaneous bacterial peritonitis.[5] Ascitic fluid infection is classified in to five categories (including three spontaneous categories) based on ascitic fluid culture results, polymorphonuclear leucocyte counts (PMN), and the presence or absence of a surgical source of infection.[4] Among five variants, SBP and culture negative neutrocytic ascites (CNNA) are common ascitic fluid infections. Prompt detection of ascitic fluid infection is helpful to use appropriate antibiotics for treatment of ascitic fluid infection. Ascitic fluid bacterial infection of cirrhotic patients may be symptomatic in 30% of cases.[6] Symptomatic ascitic fluid bacterial infection means patients who have fever, abdominal pain, abdominal tenderness either singly or in combination in patients of liver cirrhosis. Asymptomatic ascitic fluid bacterial infection means patients who have none of these symptoms and signs.[7] Positive ascitic fluid culture for bacteria was considered essential to establish the diagnosis of Spontaneous Bacterial Peritonitis (SBP). However relying on ascitic fluid culture for diagnosis of SBP has the disadvantages of poor sensitivity and relatively long time before the results are known. To circumvent problem with culture, the ascitic fluid white blood cell (WBC) and Plymorphonuclear leucocyte (PMN) counts have become the standards for making a diagnosis of SBP.[8] The aim of this study was to determine the variants of ascitic fluid bacterial infections in children with chronic liver disease.

METHODS

This present cross-sectional study was carried out enrolling 35 children, clinically suggestive of Chronic Liver Disease (CLD) with ascites in the Department of Paediatric Gastroenterology and Nutrition during the period of January, 2013 to June, 2013. Children who had features of ascitic fluid infection like fever, abdominal pain with tenderness were categorized as symptomatic and children without features of ascitic fluid infection were categorized as asymptomatic. Clinical and laboratory data of both symptomatic and asymptomatic children were recorded. Complete blood count, serum albumin, ALT, AST, Bilirubin, serum Creatinine, Prothombin time, HBsAg (ELISA), anti-HCV (ELISA), Anti LKM1 antibody (ELISA), Liver biopsy, Ultrasonography (USG) and endoscopic findings were recorded whenever necessary for selected children. Ascitic fluid was aspirated with all aseptic precautions. Thirty milliliter of ascitic fluid was collected with an 18 gauze sterile needle attached to a 50 cc disposable syringe. Ascitic fluid culture was done by conventional culture method using Tryptic Soya Broth. Ten milliliter ascitic fluid was used for culture. Five ml of ascitic fluid was sent for total WBC count, differential count and absolute neutrophil count (processed by auto analyzer and finally rechecked manually). Five milliliter ascitic fluid was sent for estimation of total protein and albumin. Demographic, clinical, haematological, biochemical and ascitic fluid study (cytology, Gram's and Ziehl Neelsen staining, total protein, albumin and culture) were done for both symptomatic and asymptomatic children. Variants of ascitic fluid bacterial infection were identified by ascitic fluid neutrophil count and culture report. Culture Negative Neutrocytic Ascites (CNNA) variety ascitic fluid infection was diagnosed by the presence of ascitic fluid polymorphonuclear leucocyte count of $\geq$250/mm^3 and negative culture report.[4] Presentation difference of both infected and non infected children were found on the basis of difference of

presenting symptoms, signs, haematological data and ascitic fluid total WBC count, neutrophil count, ascitic fluid albumin and total protein level.

RESULTS

It was observed that the age range of the children were from 2 years to 14 years and mean age was 7.39±3.0 years. The highest 48.6% (17) incidence of CLD was found in the age group of 6-10 years. A male predominance was observed in the study. Male were 54.3% (19) and female 45.7% (16). Out of 35 children, 12 (34.3%) were symptomatic i.e. they had features of ascitic fluid infection like fever, abdominal tenderness, and 23 (65.7%) asymptomatic (Table I).

Table I: Age and gender distribution of the studied subjects (n-35)

Age (in years)	Gender		Total (%)
	Male	Female	
2-5	07 (36.84%)	05 (31.25%)	12 (34.3%)
6-10	08 (42.1%)	09 (56.25%)	17 (48.6%)
11 and above	04 (21.05%)	02 (12.5%)	06 (17.1%)
Total	**19 (54.3%)**	**16 (45.7%)**	**35 (100%)**

Table II shows the difference of ascitic fluid infection in symptomatic and asymptomatic children. Out of a total 12 symptomatic children, 7 (58.4%) were infected and 5 (41.6%) were non-infected. Out of a total 23 asymptomatic children, 9 (39.1%) were infected and 14 (60.9%) were non-infected, but this difference is not statistically significant (p >0.05). So, there is no association between ascitic fluid infection and clinical symptoms.

Table II: Ascitic fluid bacterial infection in symptomatic and asymptomatic children (n-35)

Group	Symptomatic (n-12) (34.3%)		Asymptomatic (n-23) (67.7%)		χ^2-value	df	p value
	N	%	N	%			
Infected	7	58.4	9	39.1	1.15	1	0.279
Non-infected	5	41.6	14	60.9			
Total	**12**	**100**	**23**	**100**			

Table III shows range of ascitic fluid (AF) total WBC count of infected and non-infected children was 312-1200/mm^3 and 44-312/mm^3 respectively. Mean AF total WBC count of infected and non-infected children were 641.62 ± 231.99/ mm^3 and 146.05 ± 115.6/ mm^3 respectively. Range of ascitic fluid absolute neutrophil count of infected and non infected children were 255-960/ mm^3 and 10-243/ mm^3 respectively and mean ascitic fluid absolute neutrophil count of infected and non-infected children were 515±177.82/mm^3 and 85.47±70.6/mm^3 respectively. Mean difference in cell count were statistically significant.

Table III: Cytological profile of ascitic fluid (n-35)

Group	Neutrophil count/mm^3 (Mean ±SD) (Range/mm^3)	WBC count /mm^3 (Mean ±SD) (Range/mm^3)
Infected group (n-16)	515±177.82 (255-960)	641.62±231.99 (312-1200)
Non infected (n-19)	85.47±70.60 (10-243)	146.05±115.60 (44-312)
***p* value (T-value, df)**	0.001*(9.68, 33)	0.001*(3.84, 33)

*Significant

Biochemical profile (Table IV) revealed range of ascitic fluid total protein (AFTP) of infected and non- infected children was 0.7-2.8 gm/dl and 0.5-2.5 gm/dl respectively. Mean AFTP of infected 1.21±0.63 gm/dl and non infected 1.07±0.64 gm/dl, which was statistically not significant (*p*-0.05). Range of ascitic albumin of infected and non infected children was 0.2-1.4 gm/dl and 0.2-0.8 gm/dl respectively. Mean ascitic fluid albumin of infected and non-infected children were 0.56±0.35 gm/dl and 0.43±0.19 gm/dl respectively which was also not significant (*p*>0.05).

Table IV: Biochemical profile of ascetic fluid (n-35)

Group	AFTP (gm/dl) (Mean ±SD)(Range)	AF Albumin (gm/dl) (Mean ±SD)(Range)
Infected group (n=16)	1.21±0.63 (0.7-2.8)	0.56±0.35 (0.2-1.4)
Non-infected (n=19)	1.07±0.64 (0.5-2.5)	0.43±0.19 (0.2-0.8)
***p* value (T-value, df)**	0.51, (0.65, 33)	0.18 (1.35, 33)

Regarding ascitic fluid culture**,** among 35 studied children none had culture positive ascitic fluid bacterial infection, though 16 children with Culture Negative Neutrocytic (CNNA) variants had ascitic fluid bacterial infection evident by ascitic fluid neutrophil count of ≥ 250 cells/mm^3 and negative culture report. In the present study, 16 (45.7%) children had one type of ascitic fluid bacterial infection which is CNNA. Other children had no ascitic fluid bacterial infection (Table V).

Table V: Different variants of ascitic fluid bacterial infection in studied children (n-35)

Type of ascitic fluid bacterial infections	Number	%
Spontaneous Bacterial Peritonitis (SBP)	0	00
Culture-Negative Neutrocytic Ascites (CNNA)	16	45.7
Secondary Bacterial Peritonitis	0	00
Monomicrobial Non-neutrocytic (MNB) Bactericides	0	00
Polymicrobial bacterascites	0	00

DISCUSSION

In this study, most of the children were <10 years of age and the highest incidence of Chronic Liver Disease (CLD) with ascites was found in the age group of 6-10 years. The age range of the studied children was 2-14 years and the mean age was 7.39±3.0 years. In this study, male were 19 (54.3%) and female 16 (45.7%). This male predominance was also observed in another study done by Sarker[9]and Hossen[10] which was similar to the present study. Ascites polymorphonuclear cells increase with peritoneal infection or with other intra-abdominal inflammatory conditions such as diverticulitis, cholecystitis. In the present study, among the thirty five studied children, 16 children had ascitic fluid neutrophil count of ≥250 cells/mm^3 which was a diagnostic parameter of Culture Negative Neutrocytic Ascites (CNNA) type of ascitic fluid infection. In a study[9] in adult patient with Chronic liver disease(CLD) with ascites showed that, mean ascitic fluid absolute neutrophil count was 704.50±480.44 /mm^3 among infected and 56.89±45.26/mm^3 among non infected patients. Hossen[10] showed in another study in children that median ascitic fluid neutrophil count was 720/mm^3, with a range of 360-3600/mm^3 among infected children and median ascitic fluid neutrophil count was 30/mm^3 with a range of 3-192/mm^3 among non-infected children which is statistically significant (*p*-0.001). In the present study, mean ascitic fluid neutrophil count was 515±177.82/mm^3 among infected children and

mean ascitic fluid neutrophil count among non-infected children was 85.47±70.60/cmm^3 which was statistically significant (p-0.001). So, the findings of the present study in children were similar to findings of the other studies.

In sterile ascites, ascitic fluid white blood cell count is usually less than 100/mm^3 with a predominance of mononuclear cells and a low number of polymorphonuclear cells.[11] In another study done by Hoseen[10] median ascitic fluid WBC counts among infected children was 1200/mm^3 with a range of 600-10,000/mm^3 and among non-infected children it was 100/mm^3 with a range of 20-600/mm^3 which is statistically significant (*p*-0.001). In another study[10] in adult patient with CLD with ascites showed that mean ascitic fluid WBC count among infected patients was 2560±1871.91/mm^3 and mean ascitic fluid WBC count among no -infected patients was 181.85±105.30/mm^3. In the present study, mean ascitic fluid WBC count among infected children was 641.62±231.99/mm^3 and among non-infected children it was 146.05±115.60/mm^3 which was also statistically significant (*p*-0.001).

Patients with ascitic fluid total protein < 1gm/dl were the most prone to develop ascitic fluid infection and the opsonic activity of the ascitic fluid was proportional to the ascitic fluid protein concentration.[12] In a study done by Hossen, [10] mean ascitic fluid total protein was 0.36± 0.23gm/dl and 1.28±1.13gm/dl among infected and non-infected children respectively (*p*-0.087). Another study[8] in 35 adult patient with CLD with ascites showed that mean ascitic fluid total protein was 1.53± 0.61 g/dl among infected and 1.20±0.59 g/dl among non-infected patients. In this study, mean ascitic fluid total protein was 1.21±0.63gm/dl and 1.07±0.64 gm/dl among infected (CNNA) and non-infected children respectively. So, the mean value of ascitic fluid total protein of infected children of this present study is not similar with the previous study,[9] may be due to variation of age of the study populations of these two studies, though the values of the both studies are not statistically significant (*p*>0.05). Hossen[10] showed in another study in children that culture negative neutrocytic ascites (CNNA) is 16.67% In fact, the sensitivity of culture in detecting bacterial growth in neutrocytic ascites (i.e., ascitic fluid with a PMN count greater than or equal to 250 cells/mm^3) varies widely depending on the method of culture used. In published studies, the conventional method of culture has been found to detect bacterial growth in approximately 50% of neutrocytic sample.[4] In a recent study[9] in adult patient of CLD with ascites, showed that out of a total 35 patients ascitic fluid culture in conventional method showed no growth of organism, though in that study out of a total 35 patients 8 (22.8%) had PMN count of ≥ 250/mm^3. In the present study in children, out of a total 35 children, ascitic fluid culture result was found negative in all children, though 16 (45.7%) children had neutrocytic ascites (PMN count ≥ 250/mm^3). So, this study was almost similar to the study done in adult patient[9] Gene probes are now commercially available for the detection of bacteraemia; hopefully, they will lead to rapid (30 minute) and accurate detection of organisms in ascitic fluid.[14] In other study[15] in children showed that *Streptococcus pneumoniae* was isolated in 9 of 12 children suffering from cirrhosis with ascites. Another study[18] in adult patients showed that ascitic fluid culture by conventional methods was positive in 46% cases and all the culture positive cases had Gram-negative bacilli: *Escherich coli* being the most common microorganism. Ascitic fluid culture was found negative in the present study; the reason may be that the media used was not enriched enough or due to neutrophil mediated killing of bacteria.[13] On the basis of our facility only aerobic culture was done, though anaerobes can cause ascitic fluid infection rarely (1%). Most episodes of CNNA are diagnosed using insensible cultured methods, where there are insufficient numbers of bacteria to reach the threshold of detectibility.[17] The conventional method of culture probably requires at least100 organisms/ml.[13] However, even when optimal culture methods are used, a small percentage of patients grow no bacteria in their neutrocytic ascitic fluid.[18] In a study[8] in adult patients showed that out of a total 35 patients of cirrhosis with ascites 8 (22.8%) had CNNA. In the present study, among 35 children, 12 (34.3%)

children were symptomatic, i.e. they had features of ascitic fluid bacterial infection like fever, abdominal pain or tenderness and 23 (65.7%) asymptomatic, i.e. they had no features of ascitic fluid bacterial infection like fever, abdominal pain or tenderness. Out of a total 12 symptomatic children, 7 (58.4%) were infected and 5 (41.6%) were non-infected. Out of a total 23 asymptomatic children, 9 (39.1%) were infected and 14 (60.9 %) were non-infected, but this difference is not statistically significant (p>0.05). The reason of presence of features of ascitic fluid infection in non infected children may be presence of infection other than ascitic fluid infection like UTI or pneumonia etc. The reason of absence of symptoms of infection among the infected children may be due to immunosuppresion.

Limitations

The limitations of the present study were small sample size, only aerobic cultivation, and nutrient enriched media was used. Other causes of fever i.e. urinary tract infection were not excluded, so, some non-infected cases were found to be symptomatic. Many of the infected cases were asymptomatic due to immuno-suppression. This is a hospital based single centre study. Further studies with larger sample size are necessary to know the facts about the bacterial infection of ascitic fluid in children with chronic liver disease.

CONCLUSION

Culture negative neturocytic ascites variety of ascitic fluid infection was the most common variety in this current study. Polymorphonuclear neutrophil (PMN) cell count was found significantly higher in this group of children. Many of the infected cases were asymptomatic. Clinical features of ascitic fluid infection are needed to differentiate the infected and non infected cases.

Conflicts of Interest: None declared.

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