Status of Bacterial Infection in Acute Hepatitis in Different Health Facilities of Kathmandu Valley

Shrestha D.[a], Shrestha S.M.[b] & Ghimire P.[c]

Abstract

Introduction	Literally, hepatitis means infection of liver and biliary system. The most common cause of acute hepatitis in Nepal is found to be hepatitis E, which is a water-borne infection. The unknown etiology of nearly two-third acute hepatitis cases, generalized use of contaminated water, and increase in the acute hepatitis cases leading to IVC obstruction directed the researchers' interest towards undertaking this study.
Objective	To study the status of bacterial infection among acute hepatitis cases in different health facilities.
Methods	A clinical and lab-based study was conducted at Central Department of Microbiology, Tribhuvan University. The blood samples were collected from 118 consecutive patients with clinically suspected acute hepatitis attending Liver Foundation Nepal, Liver Unit of Bir Hospital, Nepal Ayurveda Hospital and Sukraraj Tropical and Infectious Disease Hospital from Aug 2001 to Aug 2002. The venous blood samples were collected aseptically from the patients and subjected to blood culture and antibiotic sensitivity testing.
Results	Bacterial infection among acute hepatitis cases was found to be 38%. Among these, 49% were gram-negative rods, 44% gram-positive cocci and 7% gram-positive rods. *Escherchia coli* (9) was the most prevalent gram-negative bacteria, followed by *Klebsiella spp.* (6), *Pseudomonas aeruginosa* (3), *Salmonella spp.* (2), *Enterobacter spp.* (1) and *Acinetobacter spp.* (1). *Staphylococcus aureus* (11) was the most prevalent among gram-positive cocci, followed by coagulase-negative staphylococci (5) and Group D Streptococcus (1). Bacterial infection was two times more in male than in female. The age group of 20-29 years was found mostly affected.
Conclusion	Bacterial infection was found to be common among acute hepatitis cases (38%), more common due to gram-negative bacilli, followed by gram-positive cocci.
Keywords	Acute hepatitis, Bacterial infection.

Introduction

The most common cause of acute hepatitis in Nepal is found to be hepatitis E, a water-borne viral infection. Acute hepatitis due to hepatitis E virus is found to be very severe and fatal. In addition, lack of quality drinking water and proper sanitation has often proved to be the cause of several outbreaks of water-borne infections in Nepal. The documented data of last few years at several hospitals reveal the frequent incidence of acute bacterial hepatitis. Some incidences of Salmonella hepatitis have presently been reported. Therefore, a possibility of bacterial infection should be taken into consideration in patients with acute hepatitis of unknown etiology. In addition to this, bacterial

infection should always be considered as a possible cause of deterioration in the clinical picture; it may be a complication of pre-existing acute or chronic liver or biliary tract disease or cause of hepatobiliary disease; prompt administration of appropriate antibiotics may save the patient's life. The prevalence of acute bacterial hepatitis has not been well documented in Nepal. Feeling the need to find out the prevalence of bacterial infection in acute hepatitis in Nepal, the study was planned and carried out during August 2001-August 2002 in Central Department of Microbiology, T.U.; in collaboration & cooperation with Liver Foundation Nepal, Tripureswor; The Liver Unit, Bir Hospital; Sukraraj Tropical and Infectious Disease Hospital, Teku; and Nepal Ayurveda Hospital, Naradevi.

Materials and Methods

The study population included clinically suspected acute hepatitis cases of all age group; attending Liver Foundation Nepal, Liver Unit of Bir Hospital, Nepal Ayurveda Hospital and Sukraraj Tropical and Infectious Disease Hospital during 2001 August-2002 August. Cases having clinical signs and symptoms suggestive of acute hepatitis with a rise in serum ALT twice the normal limit were involved in the study. The other causes of hepatitis were ruled out by careful questioning and required laboratory tests.

For bacterial culture, 5ml of patient's blood was aseptically drawn and inoculated into 50ml of sterile BHI broth in blood culture bottles followed by incubation at 37°C for up to 7 days till negative as per standard protocol. The culture bottles were examined daily for any visual evidence of microbial growth. The microscopic examination was performed for the macroscopically positive cultures. The broth culture was then subcultured on Blood Agar, MacConkey Agar, Nutrient Agar and Chocolate Agar plates. Repeated subcultures of the culture bottles were made at 24, 48, 72, 96 hours up to 7 days, till growth was negative. All the subculture plates were incubated aerobically at 37°C in an incubator and examined after 18-72 hours of incubation. Identification was made on the basis of morphological, biochemical and metabolic reactions as per standard protocols. Antibiotic susceptibility pattern of the isolates was studied using Kirby Bauer method.

Results

Out of 118 acute hepatitis cases, 83 (70%) were male and 35 (30%) were female, with male to female ratio of 2:1. Out of 118 samples subjected to blood culture, growth of bacteria was observed in 45 samples; all revealing monomicrobial infection. Higher percentage of growth (67%, 30/45) was observed among male cases compared to female cases (33%, 15/45). The overall prevalence of bacterial infection in acute hepatitis cases was found to be 38%.

Out of total samples collected from male patients, only 36% (30/83) showed bacterial growth. However, 43% (15/35) of total samples obtained from female cases were found to be growth positive. Hence, bacterial infection was found to be more common among female cases than male cases although prevalence of acute hepatitis was found to be two times more in male. The highest prevalence of bacterial infection was observed in the age group 20-29 years (33%) followed by 30-39 years (22%), 10-19 years (18%), 40-49 years (11%), 0-9 years (9%) and 50-59, 60-69, and 70-79 (2% each).

Table 1: Distribution of cases by age & gender.

Age	No. of male cases		No. of female cases		Total number of cases	
	Acute hepatitis	Bacterial infection	Acute hepatitis	Bacterial infection	Acute hepatitis	Bacterial infection
0-9	4	2	5	2	9	4
10-19	16	8	1	0	17	8
20-29	25	9	13	6	38	15
30-39	26	8	4	2	30	10
40-49	4	1	6	4	10	5
50-59	6	1	1	0	7	1
60-69	1	0	5	1	6	1
70-79	1	1	0	0	1	1
Total	83	30	35	15	118	45

Out of 45 blood culture positive samples, gram-negative bacilli were found to grow in 49%, gram-positive cocci in 44% and gram-positive bacilli in 7% samples. Infection with gram-negative bacilli was found to be the most prevalent infection in acute hepatitis. *Staphylococcus aureus* was the most common isolate (24%, 11/45) followed by *E. coli* (20%, 9/45). Among gram-negative bacilli, *E. coli* (20%, 9/45), *Klebsiella spp* (13%, 6/45), *Pseudomonas aeruginosa* (7%, 3/45), and *Salmonella spp.* (4%, 2/45), *Enterobacter spp.* (2%, 1/45) and *Acinetobacter spp.* (2%, 1/45) were isolated. Among gram-positive cocci, *Staphylococcus aureus* (24%, 11/45), coagulase-negative staphylococci (11%, 5/45), group-D streptococcus (2%, 1/45) were isolated. Beta-haemolytic *Bacillus spp* (4%, 2/45) were also isolated.

Table 2: Bacterial organisms isolated from blood culture

Bacterial organisms	Total number	%
Escherichia coli	9	20
Klebsiella spp.	6	13
Pseudomonas aeruginosa	3	7
Salmonella spp.	2	4
Enterobacter spp.	1	2
Acinetobacter spp.	1	2
Total gram-negative bacilli	**22**	**49**
Staphylococcus aureus	11	24
Coagulase-negative Staphylococci	5	11
Gr. D Streptococcus	1	2
Other GPC	3	7
Total gram-positive cocci	**20**	**44**
Beta-haemolytic Bacillus spp.	2	4
Other GPR	1	2
Total gram-positive bacilli	**3**	**7**
Total No. of bacterial isolates	**45**	

The bacterial isolates were most susceptible to ciprofloxacin and chloramphenicol (both 93.3%), followed by tetracycline (62.2%) and ampicillin (42.2%). Gram-negative bacilli were highly susceptible to amikacin (100%), followed by chloramphenicol and ciprofloxacin (both 90.9%), cefotaxime (86.4%), cotrimoxazole (68.2%), gentamicin (77.3%), tetracycline (50%) & ampicillin (40.9%). Gram-positive bacteria were most susceptible to chloramphenicol (94.7%), followed by ciprofloxacin (89.5%), tetracycline and erythromycin (both 68.4%) and ampicillin and penicillin (both 31.6%). Susceptibility of 16 gram-positive cocci (*Staphylococcus aureus and* CONS) to Methicillin was 81.2%. The isolates resistant to methicillin were further tested against vancomycin and were found to be 100% susceptible to it.

Table 3: Antibiotic susceptibility pattern of isolated bacteria.

Antibiotics	Gram-positive bacteria (#23)			Gram-negative bacteria (#22)			Total isolated bacteria (#45)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ciprofloxacin	89.5	-	10.5	90.9	9.1	-	93.3	6.7	-
Chloramphenicol	94.7	-	5.3	90.9	-	9.1	93.3	-	6.7
Tetracycline	68.4	-	31.6	50.0	18.2	31.8	62.2	8.8	28.8
Ampicillin	31.6	10.5	57.9	40.9	13.6	45.5	42.2	11.1	46.7
Amikacin	-	-	-	100.0	-	-	-	-	-
Cefotaxime	-	-	-	86.4	-	13.6	-	-	-
Cotrimoxazole	-	-	-	68.2	9.1	22.7	-	-	-
Gentamicin	45.4	36.4	18.2	77.3	4.5	18.2	-	-	-
Erythromycin	68.4	15.8	15.8	-	-	-	-	-	-
Penicillin	31.6	-	68.4	-	-	-	-	-	-
Methicillin	81.2	-	18.8	-	-	-	-	-	-
Vancomycin	100.0	-	-	-	-	-	-	-	-

S = Susceptible, I = Intermediate, R = Resistant.

Discussion

During past few years, several incidences of bacterial hepatitis have been experienced by different health personnel in Nepal. Involvement of gram-negative bacilli such as *Salmonella* in causing hepatitis, the clinical picture of which is indistinguishable from acute viral hepatitis, has been reported[1]. Overall incidence of bacterial infection in acute hepatitis cases in the present study was found to be 38%, which is relatively higher in comparison to the studies made on bacteraemia by Tribhuvan University Teaching Hospital[2]; Bir Hospital[3]; Zhao[4] and Wyke[5]. This difference may be ascribed to the difference in the type of study population, inclusion and exclusion criteria, and geographic variation. A relatively higher incidence of bacterial infection observed among acute hepatitis cases could be due to relatively greater tendency of acute hepatitis cases to contact bacterial infection. The greater tendency of bacterial infection in acute hepatitis might be

attributed to various following facts: liver is a bacterial filter[6] and the principal organ for reticuloendothelial uptake of the bacteria accounting for 60% of total uptake[7]. Therefore bacteria can infect the liver, and the liver is almost inevitably involved to some extent in all blood-borne infections[8]. However, the impairment and dysfunction of the liver in hepatitis may also facilitate the pathogenesis of bacteria[9]. The greater prevalence of acute hepatitis in males and in adult age group might be ascribed to probability of maximum exposure to the outside environment; adult age group being the most active age group and hence exposed to outside world more than the others. Various other socio-cultural and biological factors, however, might attribute to males being more susceptible than the females.

Isolation of different types of bacteria complies with the findings of Ghimire[10], Tibrewal[3] and Shakya[11]. Proportion of isolation of *E. coli* (41% of total GNR, 20% of total bacteria) is found to be higher in comparison to the findings of Wyke[5], Shakya[11], & Ghimire[10]. The incidence of *Klebsiella* infection was relatively higher in the present study than in comparison to those of Bir Hospital[3] (8.3%); Shakya[11], 2001 (5.26%) and Banjade[2]. Isolation of *Pseudomonas aeruginosa* (7%) in the present study is little higher than in the findings of Shakya[11], Banjade[2] and Tibrewal[3]. The isolation of *Salmonella spp.* is consistent with those of El-Newihi[1] and Sharma[12]. *Enterobacter spp.* accounted for 2% of total bacterial infection in the present study which corroborates with the findings of Shakya[11]. Isolation of *Acinetobacter spp.* (2%) was consistent with the finding of Shakya[11] and Tibrewal[3]. In the present study, 24% of total isolates were *Staphylococcus aureus*. This finding is consistent with those of Wyke[5], Banjade[2] and Ghimire[10]. The result, however, are not consistent with the findings of Shakya[11] and Tibrewal[3]. Isolation of *Staphylococcus aureus* from blood cultures of patients with liver abscess during 2058 has also been reported in Bir Hospital in 2002.

Conclusion

Hence, bacterial infection was found to be quite common (38%) in acute hepatitis cases. Gram-negative bacterial infection was more common compared to gram-positive infection. All the isolates showed normal antibiotic susceptibility pattern.

Acknowledgement

The authors would like to appreciate the contribution made by Nepal Health Research Council and World Health Organization by providing financial support. We are grateful to Central Department of Microbiology, Tribhuvan University for providing laboratory facility. Our sincere thanks and appreciation go to Bir Hospital, Liver Foundation Nepal, Nepal Ayurveda Hospital, Sukraraj Tropical & Infectious Disease Hospital and The Veterinary Hospital for all kinds of support.

References

1. El-Newihi HM, Alamy ME, Reynolds TB. Salmonella hepatitis: analysis of 27 cases and comparison with acute viral hepatitis. *Hepatology* 1996; **24:** 516-9
2. Banjade N, MSc Microbiology Thesis. A prospective study on aetiology of bacteraemia, septicaemia at Tribhuvan University Teaching Hospital. Submitted to CDM, 1999; TU.
3. Tibrewal A, MSc Microbiology Thesis. A prospective study on etiological agents causing infective endocarditis and related bacteraemic and septicaemic cases among patients visiting Bir Hospital. *Submitted to CDM*, 1999; *TU.*
4. Zhao JM, Wang SS, Zhang TH, Liu P, Zhou GD, Sun YL, Yin TY. Pathological and virological studies of the liver tissues from the patients with sporadic hepatitis E. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 2001; **15:** 205-207..
5. Wyke RJ, Canalese JC, Gimson AE, Williams R. Bacteraemia in patients with fulminant hepatic failure. *Postgrad Med J* 1982; **54:** 55-7
6. Filayson NDC, Hayes PG, Simpson KJ. Diseases of the liver & biliary system. *Davidson's principles & practice of medicine.* 8th Edition.
7. Churchill Livingstone. 1999; **10:** 683-736.
8. Holdsworth RJ, Neill GD, Irving AD, Cuschieri A. Blood clearance and tissue distribution of 99Tc-labelled pneumococci following splenectomy in rabbits. *Br J Exp Pathol* 1989; **70:** 669-677.
9. Betel M. Acute hepatitis B" incidence in canada. *Canada Communicable Disease Report, April.* 1997; **1:**
10. Sherlock DS. Diseases of the liver and biliary system. 7th Edition. Blackwell Scientific Publications 1987.
11. Ghimire D, MSc Microbiology Thesis. Bacteriological profile of bacteraemia and septicaemia among patients visiting Patan Hospital. Submitted to CDM, 1995;TU.
12. Shakya R, MSc Microbiology Thesis. Study on bacterial flora in blood specimen collected from hospitalized and outpatient services of Tribhuvan University Teaching Hospital. Submitted to CDM, 2001; TU.
13. Sharma V, Thakur TS, Thakur SS, Goyal A (1995).Typhoid hepatitis: report of two cases. *Gut* 1995; **37:** 136-139.