



CONCURRENT INFECTION OF SALMONELLA HEPATITIS IN LAKHIMPUR DISTRICT OF ASSAM

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ABSTRACT

Hepatitis A is a serious liver disease caused by the hepatitis A virus which occurs globally and is causing a public health concern, primarily in developing countries. In tropical endemic areas like India common mode of transmission for both enteric fever and hepatitis A are mostly through Feco-oral route. The objective of the study was to find out the source of infection as well as descriptive epidemiological analysis in terms of time, place and person during an outbreak of Hepatitis A in Lakhimpur district of Assam. 2-3 ml of blood samples were collected from suspected viral hepatitis patients followed WHO case definition. The samples were further processed for detection of Malaria (microscopic slide), Hepatitis A &E IgM (Elisa), Typhoid (Widal and Typhi dot) and Leptospira (IgM Elisa). Water samples collected from the drinking water source for H₂S and Most probable number (MPN) test. We identified 56 cases with two deaths. 48.21% of collected samples showed HAV IgM positive whereas no HEV IgM positive cases found. Likely no Malaria and Leptospira cases were detected among the suspected viral hepatitis cases. But, it was observed that 7.40% of the samples showed mixed infection of Salmonella and HAV. 91% of the collected water sample showed H₂S positive. MPN result also revealed that those drinking water source used by the people were contaminated with high quantity of bacilli and not suitable for drinking purpose.

KEY WORDS: Hepatitis A, Hepatitis E, H₂S, Leptospira, Malaria, MPN



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INTRODUCTION

In developing country like India, infectious diseases contribute maximum to the morbidity and mortality^[1]. There are many categories of infectious diseases out of which waterborne infectious diseases like diarrhea, typhoid fever, infectious hepatitis, etc. are common predicament in NE region of India. Hepatitis A, caused by infection with Hepatitis A virus (HAV) is primarily transmitted by the fecal-oral route, by either person-to-person contact or through consumption of contaminated food or water. Although viremia occurs early in infection and can persist for several weeks after onset of symptoms. There are five main types of hepatitis that are caused by a virus, A, B, C, D, and E - plus types X and G. HAV replicates in the liver and is shed in high concentrations in feces from 2 weeks before to 1 week after the onset of clinical illness^[2]. However, 10%–15% of patients experience a deterioration of symptoms during the 6 months after acute illness. Overall case-fatality rate: 0.5%. The risk for symptomatic infection is directly related to age, with >80% of adults having symptoms compatible with acute viral hepatitis and most children having either asymptomatic or unrecognized infection^[2]. Antibody produced in response to HAV infection persists for life and confers protection against reinfection. Concurrent infections in acute febrile illness can be challenging for a treating physician^[3]. Many of such infections can be water borne. Waterborne diseases are very common in India due to inadequate sewage and water drainage systems as it was accounted that multiple infections can occur with contaminated water. Few studies have shown the co existence of viral hepatitis A with other water borne diseases like Hepatitis, diarrhoea, typhoid etc. but still the diagnostic dilemma whether clinical manifestations are due to viral hepatitis or a feature of typhoid hepatitis, always challenge a clinician^[4,5]. The diagnosis of hepatitis A cannot be made on clinical grounds alone; serologic testing also is required. At the same time previous study revealed a predominance of Typhoid fever during the year 2012 in Lakhimpur district of Assam so as to bring importance of

the study^[6]. Keeping in view of the above fact a study was carried out in Lakhimpur district of Assam during an outbreak of Viral Hepatitis with clinical manifestations as well as serological confirmation to detect whether any coinfection was existing among the population. The source of drinking water and food source used by the affected people and unaffected neighbours were observed and compare cases with unaffected neighbours with respect to suspected exposure. This study help in understanding the risk factor associated with concurrent infections.

MATERIALS AND METHODS

1. Inclusion /Exclusion Criteria

Patients included in the study those followed WHO standard Case definition: Acute illness typically including acute jaundice, dark urine, anorexia, malaise, extreme fatigue, and right upper quadrant tenderness. Biological signs include increased urine urobilinogen and >2.5 times the upper limit of serum alanine aminotransferase were included in the study (Source: WHO recommended Surveillance Standards,1999). Patients who took antibiotics already were excluded from the study. Besides the above mention criteria patients of all age groups and both sexes were included.

2. Sample collection

Blood sample

2-3 ml of blood samples were collected from the patient having the symptoms of vomiting with yellowish eyes and dark urine. Patients consent was taken prior to collection of blood sample. Institutional Review board was informed about the study and permission was obtained afterwards. Patient's clinical and demographic history was taken in a predesigned Proforma. After collecting blood samples immediately serum was separated in the Laboratory at stored at -20⁰c until processing. Further Enzyme linked immunosorbent assay was performed for confirmation of aetiology of viral hepatitis. Widal test and Typhidot test was also done in all the blood samples to be acquainted with if there are any confections. All the collected

samples were also screened for the detection of malarial infection. As the study area was affected with rainy flood one month before so Leptospira IgM Elisa was also done for detection of Leptospira IgM antibody. Liver function test and kidney function test was also done in all the samples to determine the echelon of disease.

Water Sample

Water samples were collected from drinking water source of all the 65 houses of the village in a H₂S strip bottle for detection of presence of Sulphur producing bacteria. Beside this we randomly selected 30% of drinking water source and collected 500ml to 1 L water sample in a sterilized bottle for Most Probable number test.

A. HAV IgM Elisa test

(Company- DSI, Italy Sensitivity- 99 %, Specificity-98.5 % as per instruction): DS-EIA-ANTI-HAV-M-RECOMB kit is an enzyme immune assay for the detection of IgM antibodies to Hepatitis A virus in human Serum/Plasma. The kit allows to detect acute stage of the infection (2-3 Months) of patients an allows to differentiate from the late re-convalescents of Hepatitis A.

B. HEV IgM Elisa test

(Company- DSI, Italy Sensitivity- 100 %, Specificity-99.5 % as per instruction): - DS-EIA-ANTI-HEV-M-RECOMB kit is an enzyme immune assay for the detection of IgM antibodies to Hepatitis E virus in human Serum/Plasma.

C. Leptospira IgM Elisa

(Company- Scimedx Corporation, Sensitivity- 98 %, Specificity-99.5 % as per instruction): - The microwells are coated with purified leptospira coated 1 antigen, during the first incubation with the diluted patient's sera any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the samples, the enzyme conjugate is added, if antibodies have been bounds to the wells, the enzyme conjugate will then binds to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the

enzyme conjugates is present the peroxidase will catalyze a reaction that consume the peroxides and turns the chromogen from clear to blue. Addition of the stop solution in the reaction turns the blue colours to bright yellow colours. The reaction may then read visually or with an ELISA reader.

D. Microscopic slide examination for detection of malaria parasite

Thick and thin blood smears were prepared for all blood samples and stained with 10% Giemsa. The malaria parasite was detected under light microscopy. Samples were considered negative when no parasite was detected after examining 100 microscopic fields.

E. Widal and Typhi dot test

Serum was separated and screened for the presence of Salmonella through Widal test and Typhi dot test. The Widal test is applied for the diagnosis of enteric fever that includes typhoid and paratyphoid caused by Salmonella typhi and Salmonella para typhi respectively. Whereas Typhidot Rapid IgG/IgM is an immunochromatographic assay designed for the qualitative detection and differentiation of specific IgM and IgG antibodies against specific Salmonella typhi antigen in human whole blood, serum or plasma. It is intended to be used as in vitro diagnostic of typhoid fever.

F. H₂S test

The test is performed mainly to assist in the identification of Enterobacteria. The principle following: when sulphur containing amino acids are decomposed by the enterobacteria, hydrogen sulphide is produced. It reacts with ferrous ions and the formation of ferric sulphide imparts black color to the medium.

G. MPN test

The MPN test analysis is likely to enumerate fecal coliforms by observing gas production or the lack of gas production; it is possible to determine the probable number of bacteria originally present in the sample. In performing the analysis, it is necessary to have five tubes each of at least three decimal dilutions. The goal of the dilution scheme is to have some tubes positive with gas production and some

tubes negative or no gas production. The purity of the water under study with experience is determining the decimal dilutions to be used.

Epidemiological investigation pronouncement:

There was an initial case of Viral Hepatitis having the complain of vomiting with yellowish eyes and dark urine on 3rd September, 2013. The patient was attended at Kherajkhat SD on 4/9/11, Dhalpur BPHC and then admitted in NLCH (L) on 10/9/2013 and expired on 11/9/2013. Another Patient expired on

12/09/2013 at Dispur Hospital, Guwahati. Initial case followed by cluster of cases suggesting secondary spread. Till now a total of 49 (fourty nine) cases were reported from the same village with similar symptoms. The cases reached to contiguous villages and a total of 56 (fifty six) viral hepatitis cases were came into noticed under Dhalpur Block in 15 (fifteen) days from the date of onset to the ending of outbreak. The highest prevalence of infection occurred in regions where low standards of sanitation and personal hygiene among the people (Figure1).



Figure 1
Living conditions and poor hygiene in the affected village

RESULTS

Out of 56 (fifty six) sample collected, 27 (twenty seven) samples were found HAV IgM positive (Figure 2). No cases with HEV IgM positive accounted. The initial case was reported on 3rd September, 2013 and reached at peak on 14th September, 2013 and then declining (Figure 5). The last case was reported on 21st September, 2013. Case fatality rate was 4%. All age group and both the sexes were affected (Table 1). It was

observed that 7.40% of the samples showed mixed infection of Salmonella and HAV. LFT and KFT showed abnormal liver enzyme and bilirubin level. The positivity rate of malaria infection among the collected sample was found 0%. No Leptospira IgM antibody was detected among the people who suffered from viral hepatitis. Incidence of viral hepatitis was more in Bholabori Sub centre compared to Raidongia Sub centre and Jarabari Sub centre (Figure 3).

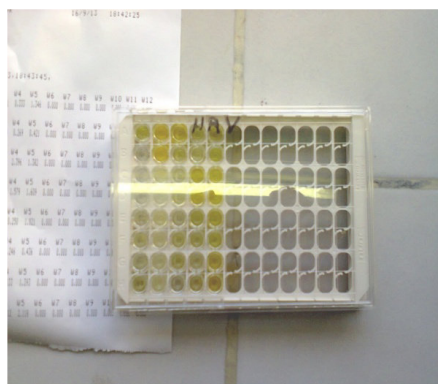


Figure 2
Elisa plate showing HAV IgM positive

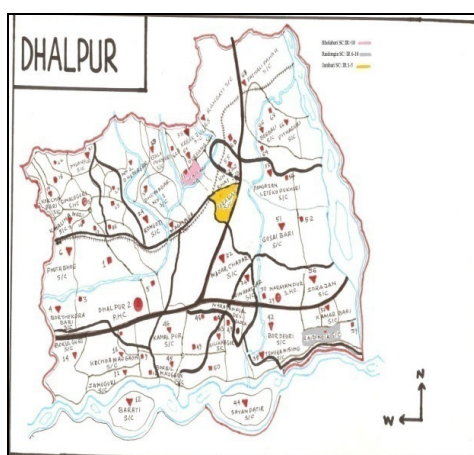


Figure 3
Map showing high incidence rate area of viral hepatitis cases (Place analysis)

65 (Sixty five) nos. of water samples were collected from drinking water source for H₂S test out of which 59 (fifty nine) nos of samples showed positive (Figure 4). MPN result showed the drinking water sources used by the villager were contaminated with bacilli (8-92 bacilli/100 ml water) and not suitable for drinking purpose.



Figure 4
Water sample showing positive result for H₂S test (black colour)

Fig 5: Number of Viral Hepatitis cases by date on onset

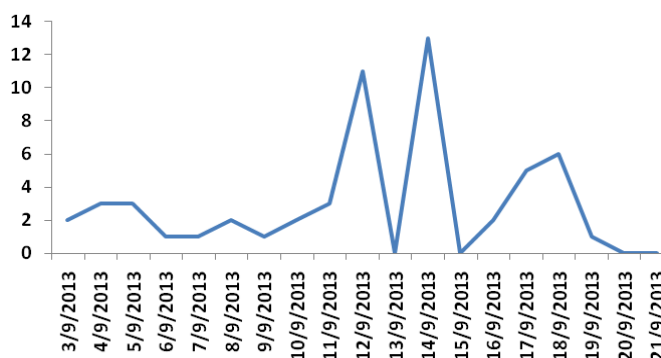


Figure 5
Number of Viral Hepatitis cases by date on onset (Time analysis)

Table 1
Number of Viral Hepatitis and HAV positive cases by age and sex (Person analysis)

Age groups (in years)	Nos of Viral Hepatitis cases	HAV IgM Positive
0 to 10	15	10
11 to 20	19	8
21 to 30	10	6
31 to 40	10	3
41 to 50	1	0
above 51	1	0
Sex groups		
Male	25	12
Female	31	15
Total	56	27

DISCUSSION

Epidemiologically, HAV is present in a worldwide distribution, the highest prevalence of infection occurring in regions where low standards of sanitation promote transmission of the virus [7]. It was a maiden report from NE India having concurrent infection of HAV with Salmonella came into observed in Lakhimpur district of Assam during viral Hepatitis outbreak. Similar finding was reported from other parts of India also [8,9]. Rate of Viral Hepatitis were in clear excess of the background in the village. We identified 56 cases with a case fatality rate of 4%. However, other reports indicated that the fatality rate in HAV infections is lower than 0.1%. Among the collected samples, 48.21% showed HAV IgM positive whereas no HEV IgM positive cases found. There was an initial cluster on 3rd September, 2013 followed by a peak around 14th September, 2013 and possible secondary spread. A newly emerged

distributary from Bisnupur near to Arunachal is passing through Garubandha bagan (Nani Bagan) bring waste harvest to a part of Bhorpur Gogoi gaon suggesting the source of infection. Although the affected village is only 2 km apart from national highway, most people were unhygienic with a habit of open air defecation so as to incidence of HAV spread through feco –oral route. Our study showed 1-20 years old age group's females are more affected. Many study revealed that higher risk is usually exposed to young children and older adults with underlying chronic liver disease [10], clinically apparent disease associated with HAV infection increases with age [11]. More than 70% of cases of HAV infection occur in children less than 6 years old are asymptomatic, or, if illness occurs, it is not accompanied by jaundice [11]. However, in older children and adults, HAV infection causes more-severe clinical illness, including jaundice malaise, fever and dark urine, in 70% of cases [12].

Beside this, Enteric fever is endemic in India with more than 300,000 cases occurring each year. The clinical presentation of Salmonella hepatitis is similar to that of viral hepatitis although certain features help in discrimination. Whereas diagnosis of hepatitis A cannot be made on clinical grounds alone; serologic testing also is required. The presence of IgM antibody to HAV is diagnostic of acute HAV infection. A positive test for total anti-HAV indicates immunity to HAV infection. In particular, fever, abdominal pain seems to be more frequent among patients with salmonella hepatitis. The study population was initially diagnosed as a case of acute viral hepatitis. Co-infection of enteric fever was suspected because of association of high fever with abdominal pain and ultimately Widal test as well as typhi dot test showed positive result. 91% of the collected water sample showed H₂S positive also confirmed the cause might be a waterborne disease. MPN result also showed the drinking water sources used by the villager were contaminated with bacilli (8-92 bacilli/100 ml water) and not suitable for drinking purpose.

Description of control measure taken

A. Active door to door search was done by the PM staffs in the affected area.

B. Medical Camps have been organized in the affected area from 13/09/2013 onwards till the control of outbreak. The cases treated locally and distributed ORS and other antibiotics to the community.

C. 24*7 hours 108 facility was provided in the village.

D. 24*7 hours Medical Camp with doctor was present at Bholabori Sub Centre which is nearest the affected village for treatment of cases.

E. IEC (awareness camp, street play) done regarding safe drinking water, hygiene and sanitation to the community.

F. LLIN mosquito net was provided in every house of the affected village. Drinking water was provided by the PHE department through tanker. 4 (Four) nos of hand pumps was installed.

The following recommendation should be

should be provided to the villagers to prevent secondary spread

- Educate the population about the risk associated with unhygienic use of wells
- Only drink commercially bottled water, or use safe and Boil drinking water if you unsure of local sanitation
- Educate for personal hygiene
- Forbid washing of cloths in wells wash your hands with soap after going to the toilet
- Only consume food that has just been cooked
- Only eat fruits that you can peel if you are somewhere where sanitation is unreliable
- Only eat raw vegetables if you are sure they have been cleaned/disinfected thoroughly
- Get a vaccine for Hepatitis A if you travel to places where hepatitis may be endemic

CONCLUSION

Co-infection of Hepatitis A with typhoid during an outbreak of viral hepatitis in some villages of Lakhimpur district of Assam with many unprotected wells and due a habit of open air defecation among the villagers. Many fraction of this district is badly affected with flood in every year. Investigation also observed short of personal hygiene among the people. In order to reduce the burden of disease vaccination against typhoid and Hepatitis A should be included in the immunization schedule.

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