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Original Article

Study of Hepatitis-E positive cases in association with presence of *E.coli* in drinking water in district Haridwar (India) region between March-June 2008

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Abstract:
Hepatitis is mainly caused by consumption of drinking water. In 1999-2000, district Haridwar, Uttar Pradesh (India) reported over 290 cases of jaundice, National references laboratory (National institute of communicable disease, Delhi) designed epidemics of water borne. In 2004, hepatitis-E, Bilkeshwar mohalla of Haridwar (India) reported cases of jaundice, again NICD proved. It was hepatitis-E common exposure among cases was the consumption of drinking water from municipal or Jal Santhan water supply. Study done during March- June 2008 from all age group patients. A total of 41 patients with hepatitis with jaundice were defined. As a person who developed acute onset of jaundice since 1st March 2008 (defined as yellow coloration of sclera). Serum sample was collected from Government Mela Hospital, Haridwar, Joint Hospital, Roorkee or during survey of hepatitis of affected area from Roorkee town. 20 samples of drinking water were collected from various sources of supply.

Introduction

Hepatitis is a general term used for inflammation of liver & can be caused by variety of different virus. Viral hepatitis is a systemic disease primarily involving the liver (A, B, C, D and E). HEV& HAV are transmitted orofecally while others through blood. All viruses cause acute or short term viral hepatitis. The hepatitis B, C and D viruses can also cause chronic hepatitis in which the infection in prolonged. Sometimes life can lead to cirrhosis, liver failure and ultimately death. Viral hepatitis causes substantial morbidity and mortality in the developing world due to both sporadic and epidemic disease (Schwartze *et al.,* 1994). Now it is a global health problem and breaks all known human carriers in causing disease (Khuroo *et al.,* 2004).

Hepatitis-E virus out breaks were mainly associated with focally contamination drinking water with hot climate water borne or enterically transmitted Non A, Non B hepatitis, is a major public health problem in India. Water becomes contaminated due to intestinal pathogens such as coli form group of bacteria Salmonellae, Vibrio & dysentery causing bacilli & sewage dumping in river & lakes. According to WHO, about 80% diseases are worldwide associated with contaminated drinking water? The primary source of pathogens has always been raw sewage and treatment of sewage for

residual microbial quality is still dream. Coli form bacteria are commonly found in soil, vegetation and surface of water. They also live in the intestine of warm blooded animals and humans. Some coli form bacteria strains can survive in soil and water for long period of times. There are 3 different groups of coli form bacteria each has different level of risk (1) total colifroms (2) fecal coliforms & (3) *E.coli*. These are all indicator of drinking water quality. Hepatitis-E virus was first identified in India and has since been recognized in middle and Far East, Northern & Western Africa, central Asian republics of former Soviet Union, China and Hong Kong SAR (Mast *et al.,* 1996). The Kathmamandu valley of Nepal is considered one of the areas of highest risk for HEV infectious (Sherstha SM, 1991, Clayson *et al.,* 1997). Most dominant cities of Hepatitis-E in India are Delhi, Ahmadabad, Kolhapur and some cities of J & K.

Theory

Hepatitis-E cases are common in rainy season when flooding leads to sewage contamination of drinking water. Presence of *E. coli* in water is strong sign of recent sewage or animal waste contamination during rain falls, *E. coli* may be washed in to river, streams, lakes or ground water, when there water are used as source of drinking water and water is not protected, treated or poorly treated, *E. coli* may get into drinking water (Corwin *et al.,* 1996).

HEV was an important cause of acute hepatitis (Jaundice) in this study. The study also revealed association of contamination of drinking water presence of *E. coli*. It is clear that HEV is closely linked to fecal contamination of drinking water in many studies. Occurrence of HEV specific IgM was noted from serum samples. It reveals that fecal contaminated drinking water as the source of HEV, it appears that rain induced flooding allows sewage to contaminate water supplies (Bradley *et al.,* 1995). All age groups were susceptible to hepatitis E infection. However adult (> 20 yrs) recorded more sero positive than adolescent and children (Schwartz et al; 1994). Hepatitis-E was not recognized as a distinct human disease until 1980, when specific tests for antibody against hepatitis-A were first applied to the study of epidemic water borne hepatitis in India. The result showed that the epidemics were not of hepatitis A, very two epidemics of water borne disease in developing countries of Asia & Africa have been linked to hepatitis A (Purcell *et al.,* 1988).

HEV is the primary cause of acute hepatitis in India, thousands cases of HEV have been reported from urban & rural areas across the Indians annually (Nayak Mc et al ;). Hepatitis-E is classified within the family Calci virus. HEV is a non envelope isocahedral, SS RNA positive sense virus (Krawzywaski, K; 1993). The prolong viremia of HEV even after the development of IgM and IgG antibodies, suggests that factors other than acute phages immunoglobulin may be important for viral clearance (Clayson *et al.,* 1995). Viral shedding in stool has been shown to begin up to 9 days priors to the enteric phase of disease (Tomar, 1998). Normally fecal shedding last up to 14 days after the onset of illness but it has been reported to continue until 7th weeks but peak antibodies titer occurring after 2-4 weeks after infection (Brayn,JP et al ;). The anti HEV IgM titers seem to decline within three months after infection during recovery phase**.** Super infection with other hepatitis viruses is also associated with more severe disease. Since multiple hepatotropic virus's infection of a single patient may amplify lever damage.

Methodology

For Serological purpose:

The following investigations were done in all cases patients:

1. Lever functioning test: serum bilirubin, SGOT, SGPT & Alkaline phosphatase.

Material: HCV, IgM, IgG, HBsAg (rapid kit), syringe, alcohol, vials, swab, band aid, tourniquet etc.

For microbiological purposes:

Two methods: 1. Hydrogen sulfide & 2.multiple tubes method (MPN), presumptive and confirmed test.

Result & Discussion:

Age specific, percentage and mean distribution of Hepatitis cases:

Age group in years	Total No. of cases	Mean of age (years)	% age
1-10	9	7.6	21.96
11-20	11	15.8	26.82
21-40	18	28.2	43.9
>41	03	46.3	7.3

Serum Bilirubin level (Mean) distribution in age specific hepatitis cases:

Age group (years)	Mean age (years)	Mean Serum Bilirubin (mg/dl)	% raised difference to normal 2 mg/dl
1-10	7.6	4.43	2.3
11-20	15.8	5.58	3.58
21-40	28.2	5.2	3.2
>41	46.3	5	3.0

Age specific raised mean of liver enzymes SGOT/SGPT:

Age group (years)	Mean age (years)	SGOT (IU)	Fold (times) increased as compare normal value 49 (IU)	SGPT (IU)	Fold (times) as compare to normal value 49 (IU)
1-10	7.6	501.88	10.2 times	330.22	6.7
11-20	15.8	952.90	19.4 times	722.09	14.7
21-40	28.2	490.22	10.0 times	416.4	8.4
>41	46.3	527.66	10.7 times	292	5.9

Anti HEV IgM sero positivity in association with sex and age in 16 randomly selected from original group of 41 cases:

Sex	Male			Female			Total (n=16)			
Age (Yrs)	Total	+ve	-ve	Total	+ve	-ve	+ve	%	-ve	%
1-10	03	01	02	0	0	0	01	6.25	02	12.5
11-20	03	02	01	02	0	0	02	12.5	03	18.75
21-40	04	02	02	02	01	01	03	25	03	12.5
> 41	02	01	01	0	0	0	01	6.25	01	6.25
Total	**12**	**06**	**05**	**04**	**01**	**01**	**08**	**50**	**08**	**50**

Anti HAV IgM, HBS Ag, HCV sero positivity in association with age and sex in 41 hepatitis cases:

Sex		Male			Female				Total +ve	otal -ve
			+ve	-ve	Total	+ve	-ve			
Age yr	Total	HAV	HBSAg	HCV		HAV	HBSAg	HCV		
1-10	07	---ve	-ve	---ve	02	---ve	---ve	---ve	00	09
11-20	08	---ve	---ve	-ve	03	---ve	---ve	---ve	00	11
21-40	12	---ve	01	---ve	06	--ve	---ve	---ve	01	17
> 41	03	-ve	--ve	---ve	00	---ve	---ve	---ve	00	3
	30	00	01	00	11	00	00	00	01	40

It is suggested that an increase of SGPT activity is more specific indicator of liver cell damage than that of SGOT **(**Wong **et al,** Khuroo, 1980 **and Panda et al;**).

In this study mean value of SGOT (501.88 IU) and SGPT (330.22 I.U) in age group of 10 yrs., age group 11-20 yrs mean value of SGOT (952.90 IU) and SGPT (722 I.U) age group 21-40 yrs mean value of SGOT (490.22 I.U) and SGPT 416.40 I.U and in more than 41 yrs SGOT (528.66) and SGPT (292I.U) mean value. Elevation of SGOT and SGPT highest in age group 11-20 yrs in all age groups SGOT in more elevated as compared to SGPT almost similar range of elevation found in children below the 10 yrs. In adult age <20 yrs SGOT 19 times increased (normal range 49 Iu) and SGPt 14 times increased it found highest rest all age group shows SGOT more than 10 times SGOT 5 to 8 times. Study shows serum enzymes in Hepatitis E is raised > 8 times similar finding in the present study in human levels of liver enzymes and indicators of liver inflammation SGOT and SGPT rise dramatically 4-5 weeks after infection and remain elevation for 20-90 days (Fletcher J. 1993). The liver is probably main site of HEV replication in infected Human. All 41 cases were found serologically negative for HBSAg and anti HCV except one adult age 45 years positive for HBSAg 2%. 40 cases (98%) were potential cases of Non-A Non-B hepatitis. Randomly selected 16 samples from original group were tested for presence of Anti HAV IgM, Anti HEV IgM and Anti HCV IgM

All 16 samples were found negative for anti HAV IgM, anti HCV IgM only one male age group 21-40 years found positive for HBSAg. So hepatitis cases in the present work were Non-A Non-B there sub set of 16 cases (39%) of original group of 41 were tested for presence of Anti HEV IgM antibodies.

In age group 1-10 yrs, 1 case (6.25%) male were positive age group 11-20 yrs 02 cases (12.5%) male were positive age group 21-40 yrs 03 cases (25%) 02 male and one female were positive for anti HEV IgM antibodies indicating that these hepatitis cases represents recent infection with HEV. Maximum age in this study > 41 yrs case was found positive for anti HEV IgM antibodies. Thus All 41 samples were negative for Hepatitis A, clinical hepatitis A is uncommon among the adult population in western India. Because of seroconversion to HAV early in childhood (Aran Kalle *et al.,* 1992).

In this there were 22% acute hepatitis cases without any serological marker of acute viral hepatitis A, B, E and C. However the absence of anti HAV IgM, anti HBV core antigen IgM, Anti

HCV IgM and Anti HEV antibodies in approximately 22% of the patients with symptoms of acute hepatitis suggest that there may be a yet unidentified virus in the area or sample may be collected earlier no antibodies raised against the virus antigen.

Currently three methods are routinely used to detect *E. coli* organisms in water : presences – absence which is a qualitative test membrane filter (MF) and multiple tube fermentation (MTF) which as both quantitative tests in this study MPN test show 6 (30%) samples were excellent no presence of *E. coli*, 3 (15%) satisfactory, 5 (25%) suspicious only 6 (30%) were unsatisfactory.

Twenty water samples were collected at different points for MPN count one for each samples taken in H_2S vials for rapid results, 50% samples were found no color change means no organisms present in drinking water only 9 samples 45% changes color yellow with color indicating presence of organism (*E. coli*) only one (5%) sample change color in Black, indicating presence of organism like salmonella or citrobactor.

Conclusion

The study revealed many typical characteristics of HEV reported in the literature, provided evidence for HEV agent and to be spread by fecal contamination. Contamination of drinking water find by indicator organism *E. coli*. Further studies will be needed to determine where the HEV is associated with presence of indicator organism *E. coli* in drinking water. In distt Haridwar 1999-2000, observed a large number of hepatitis cases; National Institute of Communicable Disease confirmed the HEV infection and occurred out break due to contamination of drinking water earlier studies conducted in other parts of India have also provided evidence for existence of a HEV hepatitis agent, which would be the agent to be spread by fecal contamination. This study carried out at Intergraded Disease Surveillance Laboratory Distt Haridwar on 41 cases who presented with the symptoms of Jaundice (PILIA) 20 water samples were collected, different sources from the area of Jaundice cases, analyzed bacteriological by MPN method, to establish the presence of *E. coli*. All cases were investigated for liver function test and screened serologically for hepatitis causative agent hepatitis E, A, B and C viruses by detecting IgM antibodies. Drinking water investigated for presence of E-Coli by H_2S (Rapid Method) and multiple tube method (MPN).

We find out all age group were susceptible to hepatitis infection, however adult >20 yrs (43.9%) recorded more in comparison to adolescent (26.82%) and children 21.96%. Male contribution is almost ¾ of cases, perhaps due to adult male are more exposed to risk factors of getting infection.

Liver infections Serum Biliburin raised high in age group 11-20 yrs than age group 21-40 yrs it was lowest below the 10 yrs of age, in comparison to male, more raised in males, perhaps due to physical activity of male.

Liver enzymes SGOT (Serum Glutamic Oxaloacetate Transamine) and SGPT (Serum Glutamic Pyruvate Transamine) elevation of SGOt and SGPT highest in age group 11-20 yrs in all age groups SGOT in more elevated as compared to SGPT. Almost similar range of elevation found in children below the 10 yrs. in adult age < 20 yrs SGOT was raised 19 times and SGPT 14 times as compared to normal range. In human enzymes levels are indicators of liver inflammation and liver damage. Probably liver cells are main site for replication of hepatitis E virus in infected human. SGPT in more raised in males than females. It is suggestive of more liver damage in meals. Because SGPT is more specific indicator of liver cell damage.

In this study, find that all age group were susceptible to hepatitis E, however adult > 20 yrs

more sero positive than adolescent and children in age group 21-40 yrs 25% were positive for hepatitis E virus. (Anti HEV IgM antibodies). Presence of anti HEV IgM antibodies represents recent infection of HEV. All 41 serums were negative for hepatitis A these were 22% of Jaundice cases without any serological marker of HAV, HEV, HBV and HCV. However the absence of IgM antibodies against their agent may be in identified virus or sample may be collected earlier, no antibodies raised against the virus antigens.

Drinking water samples analysis for presence of E-coli by multiple tube method:

Mean of most probable no. of coliform/100 ml	Total sample	% of sample	Quality grade of drinking water
0	6	30	Excellent
2 (2.3)	3	15	Satisfactory
8 (6.8=7.0)	5	25	Suspicious
25	6	30	Unsatisfactory

Conformation of E-Coli in unsatisfactory & suspicious water samples:

Water quality	Total No.	Sample Shows growth on mac conlay agar	No. growth
Unsatisfactory	6	5	01
Suspicious	5	3	02

Twenty water samples were collected at different points for MPN count each sample also tested for H_2S rapid method for presence of indicator organism *E. coli* samples for H_2S test, 50% samples were found no color change means no organism present in the sample 45% samples shows color change yellow with color indicating presence of organism like *E. coli* only 5% sample color change in black, indicating other organisms other than indicator organism. All water samples also set for MPN test 30% water samples found excellent no organism present 15% were satisfactory means less than 3 *E. coli*/100ml (probable number) present means water suitable for drinking or potable water 25% suspicious means 4 to 10 *E. coli*/100ml (probable number) and 30% were un satisfactory means presumptive coliform count >10/100ml it is indicating that there is contamination of drinking water number of hepatitis cases are also common in that area. Presence of *E. coli* in water is strong sign of recent sewage or animal waste contamination either due to blockage of sewage line or leakage in water pipe line system, there are many on line booster pumps in affected area that is why there is chances of back sucking of dirty water. Broken or cracked piping, as has been observed in the affected area, negative pressure can pull in fecal contaminated water from the surface above the pipes, these by increasing the risk of HEV contamination.

In this study revealed that hepatitis E virus infection was water borne and associated with contamination of drinking water. Presence of indicator organism *E. coli* contamination of drinking water and association with HEV.

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