Indian Journal of Microbiology Vol 36, September 1996, pp 167-168.

Short Communication

Detection of Anti-HAV-IgM Marker and HBV-DNA in Sera from Children with Acute Jaundice

S, Ananthan*, M Ananthasubramanian, P Saravanan and SV Alavandi
Department of Microbiology, Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences, Taramani, Madras 600 113 **India.**

An investigation was conducted to understand the frequency of co-infection of hepatitis A virus(HAV) and hepatitis B virus (HBV) in children below 10 years of age with clinically confirmed acute jaundice. Serum samples from 18 children were screened for anti-HAV-IgM by ELISA and HBV-DNA using Dot blot hybridisation technique to find out the association of HBV infection in these cases. Anti HAV IgM marker was detected in 11 cases. HBV-DNA was detected in 4 sera samples, 2 of which were also positive for anti HAV-IgM.

Key Words: Hepatitus A virus, Hepatitus B virus, Jaundice, IgM antibodies

Hepatitis A virus (HAV) has been the most common cause of acute hepatitis in children. Exposure to HAV occurs in early childhood and by the age of 10 years, 90% of healthy persons have serological evidence of HAV infection (1). Occasionally, HAV has been recorded as an etiological agent in epidemics of viral hepatitis (2). Hepatitis B Virus (HBV) is responsible for most of the chronic hepatitis cases in childhood. The chronic carrier state is acquired by early horizontal or vertical contact among children (3). It has been recorded that the course of HBV infections in children is most often mild, and in children younger than 2 years of age it is usually inapparent (4). Since the primary immune response in acute HAV infection involves IgM antibodies, demonstration of this class of antibodies with specificity to HAV has offered a valuable method for the rapid diagnosis and also to detect recent infection. This marker is also useful for (exclusion-based) diagnosis of enterically transmitted non-A non-B hepatitis (5). Since prognosis and management of hepatitis in children depends on the specific causative agent, it is important to screen other causative factors, particularly HBV, when children show symptoms of hepatitis (6). The present study was undertaken to understand the frequency of acute HAV infection among children with acute jaundice and to find out the association of HBV in these cases.

Blood samples were collected from chidlren with 1-10 years of age group admitted for acute jaundice at the Institute of Child Health & Hospital for Children, Madras. All the sera were screened for anti-HAV IgM marker and HBV-DNA at the Molecular virology unit, University Department of Medicine, Royal Free Hospital, London. Bio ELISA HAV-IgM (BIOKIT, SA SPAIN), a commercial test kit was used to screen the anti-HAV-IgM. The test procedure was followed as per the instructions of the manufacturer.

For detection of HBV- DNA by dot blot hybridiztion, 50 µl serum sample was digested by treating with 20 µl of a detergent NP 40 (10%) and 20 µl of mercapto-ethanol (3%). The mixture was kept at room temperature for 5 min and 90 µl of 2 M solution of NaCl and 190 µl of 1M NaOH was added for denaturation. From this mixture 200 µl was used for dot blot hybridization assay on nitrocellulose paper in a dot blot apparatus.

HBV-DNA was detected by dot blot hybridization and autoradiography using a ^{32}P labelled (7) HBV-probe according to the method described by Scoto *et al.* (8). Appropriate HBV positive controls ranging from 4 ρ g to 200 ρg were incorporated in the assays. Quantifi-cation of the HBV-DNA in the samples with reference to the cloned standard HBV-DNA was done by densito-metry of the autoradiograph.

The present study has clearly established that 11 out of 18 (61%) of the children have been exposed to recent infection of HAV as evidenced by anti-HAV-IgM positively through ELISA and 4 out of 18 (22%) were found to have HBV-DNA ranging from 0.1 to 0.3 ρg ml^{-1} of the serum as detected by dot blot-hybridization (Table.1). Two sera samples positive for HBV-DNA were also positive for anti HAV-IgM. Three cases of hepatitis could not be attributed to any of these etiologies.

Among the study group, out of 9 children under 5 years of age, 8 were found to be positive for HAV-IgM marker and none were positive for HBV-DNA. In the age group of 5-10 yeares, anti HAV-IgM marker and HBV-DNA were detected among 3 and 4 children respectively, 2 exhibited positivity for both HAV-marker and HBV DNA. Elevated levels of bilirubin was observed in the serum samples among children with anti-HAV-IgM and HBV-DNA positivity. It is well known that more than

* Corresponding author

80% of the children acquire natural infection by the age of 5 years to HAV (3). In the present study, we have not looked into the anti-HAV-IgG marker to know the past infection. Previous research has indicated that in 80-90% of cases, IgM-antibodies are detectable for 3 to 6 months after the onset of illness, whereas IgG-anti-HAV can persist indefinitely. As IgM-anti-HAV are produced transiently, its presence in sera indicates ongoing or recent infection and is the most valuable test for acute hepatitis A (1,4). In children, HAV is usually asymptomatic, while in adults, symptomatic infection is characteristic and jaundice is common. The onset of HAV is often abrupt and characteristic prodromal symptoms are followed within a few days to a week, by dark urine and jaundice (9).

Table:1. Anti-HAV-IgM and HBV-DNA positivity status among children.

Age group	No. screened	No. of sera positive for		Total bilirubin levels (mg ml^{-1})
		Anti-HAV -IgM	HBV-DNA (Conc. ρ ml^{-1})	
1-2	2	2	-	10.9, 3.0
2-3	3	3	-	5.0, 5.5, 4.9
3-4	1	1	-	4.8
4-5	3	2	-	2.6, 2.6
5-6	2	1	1(0.3)	8.0
6-7	2	-	2(0.2, 0.12)	5.7, 7.7
7-8	2	-	-	-
8-9	1	1	-	3.4
9-10	2	1	1(0.1)	5.7

Nucleic acid hybridization assays with DNA probes play a major role in the identification of hepatitis B virus and have proved essential for monitoring the course of disease and evaluating treatment (8, 10, 11). HBV-DNA in serum has been reported as a valuable marker for evaluation of viral replication in HBsAG (Hepatitis B virus surface antigen) positive individuals (10). Analysis of serum HBV-DNA has proved to be the ultimate test in discrimination between highly infectious and weakly infections or non-infectious HBsAG positive individuals (8, 11, 12). Detection of HBV- DNA in sera by the DNA hybridization methods has been shown to be more sensitive and is a more direct *in vitro* test for assessing HBV infectivity, when compared with other HBV serological markers (8, 10, 12). Further it is also reported that HBV DNA could be detected even in the sero negative healthy individuals (13).

HBV infections acquired during early childhood are likely to progress to chronicity. It has been demonstrated by Uchaikin *et al* (15) that, HAV acounts for 83.1%, acute HBV for 11.1%, HBV and HDV(hepatitis delta virus) coinfection for 2.6% and viral hepatitis non I-A, non I-B for 1.7% among the children affected with viral hepatitis.In the first year of life, acute hepatitis was eliologicaly related to HBV and HDV co-infection. HAV was diagnosed in the presence of chronic HBV infection (1.2%) or exacerbation of chronic hepatitis B (0.3%).

In the present study, among 18 children screened, 11 were positive for HAV inclusive of two cases with HBV infection, and 2 cases positive for HBV alone. Three cases were negative for both HAV and HBV, which could be possibly grouped under hepatitis due to non-A non-B viral infection. Further, screening of HBV positive sera for IgM anti-HBV would have indicated acuteness of hepatitis B infection. This study has indicated coinfection of HAV and HBV, and such information would be useful in management of viral hepatitis problem in childhood.

The help of Dr. Tim Harrison in conducting this piece of work at Molecular Virology unit, University Department of Medicine, Royal Free Hospital, London is gratefully acknowledged.

References

1. **Tandon BN, Gandhi BM & Joshi YK,** *Bull WHO,* **61** (1984) 76.
2. **Joshi YK, Tandon BN & Gandhi BM,***Indian J. Med Res,* **81**(1988) 96.
3. **Deinhardt F & Gust ID,***Bull WHO* , **60** (1982) 661.
4. **Mierzeiewska RA and Sale E.,***Epidemiol*, **47**(1993) 243.
5. **Chitambar SD, Arankalla VA & Banerjee K,** *Indian J Med Res,***93** (1991) 19.
6. **Hadchouel M Rev Prat, 40**(1990) (18) 1667.
7. **Feinberg & Vogelstein,** *Anal Biochem,* **132**(1983) 6.
8. **Scotto J, Hadchouel M, Hery C, Yuvat J,** *Tiollais P & Brechot C Hepatol,* **3**(1983) 279-284.
9. **Koff RS,** *Vaccine,* **10**(1992) (suppl.) 15.
10. **Bonino F, Hoyer B, Nelson J, Engle R, Verme G & Gerin JL,** *Hepalot*, **1**(1981)386.
11. **Hadziyannis SJ, Lieberaman HM, Karvountzis GG & Shafritz DA,***Hepatol,* **3**(1983) 656.
12. **Vantine Thon E, Steinmann J & Arnold W** *Mol. Cell Probes*, **5** (1991) 299.
13. **Pao CC, Yao DS, Lin CY, Kao SM, Tsao KC, Sun CF & Liaw YF,** *Am J Clin Pathol* , **95**(1991)591.
14. **Grosheide PM, Rothbarth PH & Gerards LJ,** *Tijdsch Kindergeneeskd,* (Dutch) **58**(1990) 203.
15. **Uchaikin VF, Kaganov BS, Levina EI, Chaplygina GV, Sizykh NN, Konev VA, Stepanov AN & Sergeev Siu,** Pediatria, **1**(1991)8