

### HEPATITIS C VIRUS (HCV) INFECTION IN PEDIATRIC PRACTICE

Soon after serological markers of hepatitis A and B viruses were available in the later 1960's, it became clear that there was a sizable group of hepatitis patients, who have infection due to other viral agents, which were termed by Alter *et al.* in 1975 as 'non-A non-B hepatitis'(1). Major breakthrough was achieved when the identification of a cDNA clone, 5-1-1 which encoded at least one epitope that specifically identified antibodies in serum from patients with blood-transmitted NANBH was reported by scientists at Chiron Corporation California(2). By using a hybridization technique, a large overlapping clone, C 100-3 producing a non-structural protein was constructed(2). This clone was derived from a virus which was named hepatitis C virus (HCV). A first generation antibody test for diagnosing HCV (anti-HCV) was developed(3). Over a short period thereafter HCV research progressed rapidly in several directions, viz., molecular basis, diagnostic testing, transmission and therapy.

#### Viral Characteristics

HCV is a single stranded RNA virus of flavivirus family. Its genome comprises of 10,000 nucleotides in a single open reading frame. The structural codons (C) are located in the 5' end and non-structural codons (NS<sub>1</sub>-NS<sub>5</sub> in 3' end. Between these

two regions is a hypervariable region (E<sub>1</sub>, E<sub>2</sub>NS<sub>1</sub>) encoding for the envelope protein (Fig). Subsequently, a few HCV variants have also been identified(4).

#### Diagnosis of HCV Infection

Development of serological tests are based on the knowledge of viral genome. First generation anti-HCV antibody test was the initial serological test used for diagnosis(3) synthesized in yeast by recombinant DNA technology. Encoded by non-structural region (NS<sub>3</sub> and NS<sub>4</sub>) of HCV genome is fused with human superoxide dismutase which facilitate efficient expression of foreign protein in yeast and bacteria. After solubilization and purification, C-100-3 is used to coat the wells of microtitre plates so that circulating HCV antibodies can be captured and measured by the use of second radioactive antibody. False positive results with first generation assay were observed in patients with hypergammaglobulinemia, autoimmune type I chronic active hepatitis, rheumatoid arthritis, malaria, paraproteinemia, etc. Prolonged storage, repeated freezing and thawing were considered to be other causes of false positivity. As superoxide dismutase is a component of C-100-3 the test shows cross reactivity to superoxide dismutase(5). In second generation tests, three antigens—one encoded by NS3 region (C33), one from structural region (C22) in addition to C-100-3 are used in nitrocellulose strip method. Sample is considered positive if it is reactive to at least two of the three antigens. Both first generation and second generation enzyme linked immunosorbent assay (ELISA) and radio immunoblot assay (RIBA) are available com-

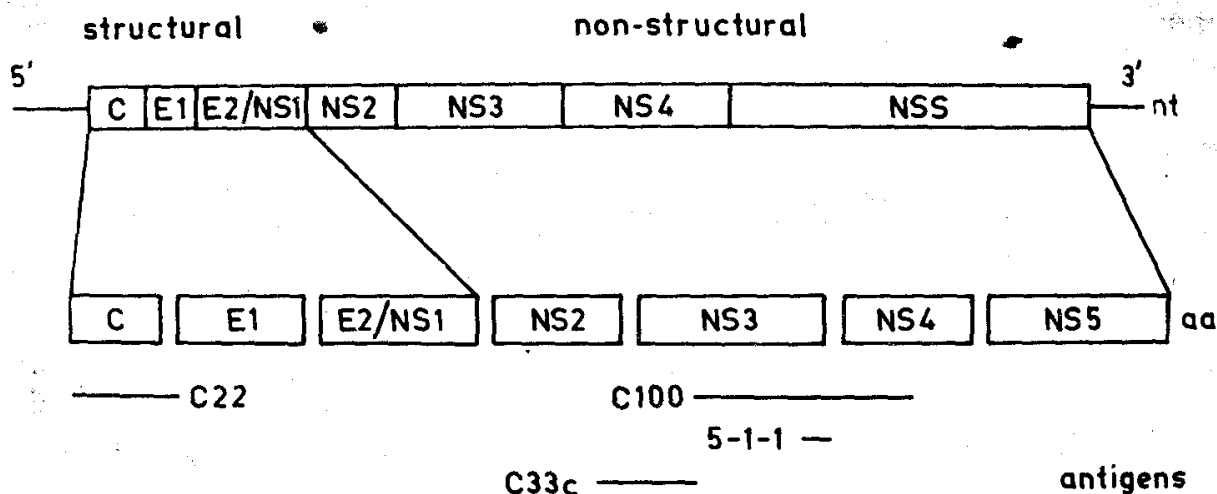


Fig. Schematic representation of hepatitis C virus genome. The relative location of individual viral proteins C22, C33, C100 and 5-1-1 are shown.

mercially. Second generation tests are superior to first generation tests because of lower frequency of false positive reactions, higher sensitivity, shorter period of window phase before seroconversion in acute HCV infection (mean of 2.3 weeks with second generation vs mean of 6.1 weeks with first generation) and higher predictability for ongoing viral replication(5,6). Recently, detection of HCV RNA in serum by polymerase chain reaction (PCR) has been established as gold standard for diagnosis. PCR has been used for comparing sensitivity and specificity of first and second generation tests(6), for studying vertical and horizontal transmissions of HCV(7,8), and also to assess the response to antiviral therapy in chronic HCV infection. Very recently HCV core antigen has been detected by polyclonal anti-HCV antigen probes by immunofluorescence in hepatocytes(9).

### Hepatitis C Virus Infection in Children

Studies on HCV are relatively sparse and are mainly restricted to those on modes of transmission and prevalence

among thalassemic and hemophiliac children.

**Modes of Transmission:** Parenteral transmission is the major route of transmission. HCV accounts for about 95% of cases with hepatitis among recipients of blood transfusion(4). Transplacental transmission has also been conclusively proved. Seven of the eight children born to eight HCV antibody and HCV RNA positive mothers were positive for HCV RNA by PCR(7). Other less important modes of transmission are sexual, accidental needle prick exposure and organ transplantation in adults(8). Sporadic varieties of acute and chronic hepatitis C are also known to occur(4,11).

**Groups at Risk:** Children at high risk include those who require multiple blood or blood product transfusions for diseases like thalassemia, hemophilia, gastrointestinal bleed due to portal hypertension, leukemia(12), chronic renal failure on maintenance dialysis, etc.(13). Prevalence of anti-HCV antibody (using first generation tests) among healthy blood donors varies from

0.4 to 1.4% in different countries(14,15). In a report from India prevalence of 1.14% was found(17). A very high anti-HCV positivity of 14.3 to 17% was found among Indian thalassemic children receiving chronic blood transfusion therapy(18-20). NANB hepatitis has been implicated as a cause of cirrhosis in 25% of thalassemic patients receiving blood transfusions(21). Likewise, the frequency of anti-HCV positivity varies greatly ranging from 25 to 82% in multitransfused hemophiliacs(18,22). The prevalence was higher in patients receiving more than 10,000 units of Factor VIII concentrate per year and particularly those who received unmodified concentrate than dry heated concentrate(22). Either chronic active hepatitis or cirrhosis was observed among 20% of multitransfused hemophiliacs(23).

*Clinical Importance:* The incubation period of HCV varies between 2 to 26 weeks (mean 7.8 weeks). Majority of patients following acute infection remains asymptomatic or are mildly symptomatic; only 10-15% develop icteric illness(4). Half of the patients develop chronic liver disease and are observed to have characteristic fluctuations of transaminases. Progression of liver disease is slow and symptomatic cirrhosis develops over 10-15 years(24). Data on HCV infection in children are sparse. HCV positivity was found in 4/6 children with acute and chronic NANB hepatitis and also in patients with HAV and HBV infections suggesting possibility of co-infection(11). In the study of 24 children with liver disease anti-HCV was present in 21% highlighting the importance of HCV in pediatric population in India(25). Multitransfused patients of extrahepatic portal vein obstruction are also at higher risk of developing HCV infection(25). It is important

for all pediatricians to remember that HCV leads to hepatocellular carcinoma in adults(26).

### Treatment

Several drugs with established anti-viral effect like interferon (IFN), ribavirin, inosine pranobex and acyclovir have so far been tried in the treatment of HCV related liver disease. Of these, IFN has been widely used since it was approved by US Government Agency for human use on a fast track. Cumulative data in adults, using IFN therapy in the doses of 1-3 m $\mu$  thrice weekly for 6 to 9 months result in normalization of liver enzymes in 50% of treated patients. Unfortunately, 50% of these patients relapse within 6 months of stoppage of therapy; thus only one of four treated patients showed remission over a follow up period of one year. Side effects of IFN therapy reported are flu like syndrome, bone marrow suppression, alopecia, myalgia, fatigue, thyroiditis, hemolytic anemia, etc. These occur in 10-20% of cases, needing dose reduction in 15% cases and drug withdrawal in 5% patients(5). Treatment with recombinant IFN alpha (3 m $\mu$ /m<sup>2</sup> three times per week for 6 months) in 12 children with chronic hepatitis C normalized ALT levels in 38% patients at 6 months, 90% at 15 months and 45% at 24 months. Reduction of HCV RNA level and improved liver histology were also shown(27). IFN therapy shows unfavorable response in cirrhotic states. Trials of IFN therapy in adults with acute hepatitis C resulted in improvement and thus prevented progression to chronicity(28).

### Conclusion

Since the discovery of the HCV genome in 1989, phenomenal progress has

taken place. Despite intensive work in this area, sparse data are available in pediatric population in world literature in general and in India in particular. Only a few articles related to HCV in children have so far been published from India(18,20,25). Paucity of work from our country appears primarily to be due to non-availability of diagnostic kits in most of the centres (due to cost factor), lack of awareness and use of diagnostic tests in appropriate settings. It is important, therefore, to direct our research efforts towards knowing the frequency of HCV infection among blood donors, patients of post-transfusion and sporadic varieties of NANB hepatitis (acute and chronic) and cirrhosis. Such efforts will help us in framing policies of prevention and setting therapeutic guidelines. Prevalence studies can be best undertaken using second generation anti-HCV testing kits. Interferon therapy (3 m $\mu$ /m<sup>2</sup> thrice a week for 6 months) will presently cost approximately rupees sixty thousand per patient. Factors of high cost and low long term effectiveness of this therapy will probably limit its use in Indian children. More emphasis, therefore, needs to be put on prevention which can be achieved by taking several measures. The present day policy of frequent use of transfusions in children in our country needs to be discouraged actively and a judicious selection of patients for transfusion should be adopted. Secondly, policy of universal HCV screening of donor blood would significantly reduce the chances of HCV transmission among the recipients(20). In high risk situations like multi-transfused hemophiliacs, future efforts in India may include chemical or thermal sterilization of blood products(29). Well controlled pilot follow up studies should be planned in our country to assess costs

and benefits of vigorous measures of donor blood screening and sterilization of blood and blood products for HCV infection.

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