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Hepatitis B, C & D Viral Markers in Multitransfused Thalassemic Children : Long Term Complications and Present Management

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Blood transfusion at regular intervals to maintain hemoglobin above 10 gm/dl along with daily chelation therapy remains to be the main therapeutic management in thalassemia.[1] These thalassemic children are at higher risk of developing transfusion transmitted infections, which include hepatitis B, C, D, HIV, cytomegalovirus, malaria, syphilis etc. Blood banks in this country are unable to screen the donors for all types of transfusion transmitted infections due to cost prohibition, lack of organization, awareness and many other technical factors. Thus, in the prevailing circumstances of inadequate donor screening strategy in this country, information on the dynamics of hepatotropic viral infection amongst multitransfused individuals assumes an importance to devise a rational long term approach in the management of such individuals. With the advent of serological markers of various hepatotropic viral infection, it is possible at present to assess the magnitude, natural course and therapeutic response of such patients.

The present review deals with serological changes in hepatitis B, C and D, along with the prevalence, the natural course and the current management in multitransfused thalassemics.

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Hepatitis 'B' Virus Serology

Hepatitis B virus (HBV) is a medium size DNA virus and its infection in humans manifests as acute or chronic hepatitis. Its transmission is mainly through parenteral route. Acute hepatitis B virus infection is often self-limiting and only 10% of the patients, subsequent to acute HBV infection, develop chronic sequelae like chronic hepatitis and cirrhosis" Serological profile subsequent to acute and chronic hepatitis B infection is shown in Figs. 1 and 2. Hepatitis B surface antigen (HBsAg) from HBV envelop is detected in serum both during acute and chronic hepatitis.[2] Presence of HBsAg is indicative of active HBV infection. However, its presence or titer neither suggests acute or chronic HBV infection nor the severity (mild or severe) of hepatitis. Some patients may even have HBsAg in high concentrations and are perfectly healthy and are termed as carriers of HBV.[3] HBeAg is secreted from nucleocapsid gene of HBV.[4] It is found during acute and chronic hepatitis and is not detected in the serum in the absence of HBsAg. Presence of HBsAg along with HBsAg is suggestive of viral replication and active liver disease. HBcAg (Hepatitis B core antigen)

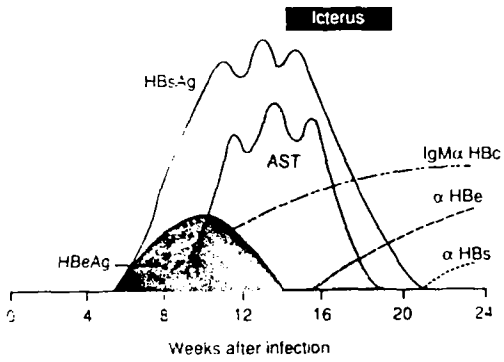

Fig. 1. Serology in acute hepatitis B virus infection.

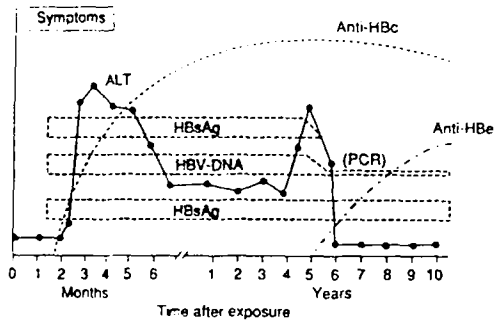

Fig. 2. Serology in chronic hepatitis B virus infection.

is not detectable by conventional ELISA/RIA techniques. However, it can be detected in the liver by immunohistochemistry and serves as a sensitive mean for detecting ongoing viral replication.[5,6] HBV DNA is also detectable in serum during acute and chronic HBV infection.[5,6] It can be detected by using direct "blot' or liquid hybridization assays. Recently PCR technique has been used for detecting HBV DNA in the serum and serves as the most sensitive method, as the HBV DNA in level of 10 pg/ml can be detected easily.[7] HBV DNA can be detected practically in all the patients who are positive for HBsAg and even before HBsAg appears or after it disappears. Detection of HBV DNA by "blot" technique is of the same clinical significance as that of the detection of HBsAg and HBeAg which is currently used to assess the outcome of treatment with antiviral agents.[8]

Anti-HBs develops after the disappearance of HBsAg and during convalescence from hepatitis. It presence is indicative of recovery and immunity against HBV infection.[9] Successful vaccination also induces anti-HBs but the level of protection is variable. It is of interest that some chronic HBsAg carriers have both HBsAg and anti-HBs which is possible only when the antibodies are directed against HBsAg epitopes and not shared by the circulating antigen.[10] Anti-HBs these people are not protective.

IgM anti-HBc is detected at the onset of acute hepatitis and persists for 3 to 12 months if the acute hepatitis resolves.[11] However, if the patient develops chronic hepatitis B, the Igm anti-HBc may persist as long as viral replication persists.[12] Igm anti-HBc appears with the onset of symptoms and rise in transaminases level. Its presence is suggestive of previous or ongoing infection with HBV as its level persists for long periods.[13] It serves as a good tool for the epidemiological studies to determine the prevalence of present or past HBV infection. IgG anti-HBc does not appear following HBV vaccination. Anti-HBe becomes detectable when HBeAg disappears during acute or chronic hepatitis B. Appearance of anti-HBe indicates the resolution of underlying infection, except when a precore HBV mutant is causing hepatitis.

Hepatitis C Virus Serology

Hepatitis C virus (HCV) is a RNA virus identified in 1989 and is responsible for majority of transfusion transmitted non-A and non-B hepatitis.[14,15] Serological tests for HCV are available but are still in the process of development. Presently only anti-HCV is commercially available.[16] Initially anti-HCV recombinant yeast polypetide (C 100-3) derived from a non-structural region of HCV genome was used. As the antigen was against a small HCV genome, there was high false positivity. No, several recombinant proteins from various regions of HCV genome have been developed for testing anti-HCV. Use of these antigens have increased the specificity and sensitivity of the test. These IInd/IIIrd generation anti-HCV assays do not distinguish between Igm and IgG antibodies. Anti-HCV is detected late during the course of acute hepatitis (between 4 to 24 weeks) and it disappears over years. While in chronic hepatitis C, anti-HCV persists indefinitely (Fig. 3). HCV RNA can only be detected by PCR technique as the virus circulates in very low levels. Majority of the patients with acute hepatitis C, 40 to 75% of the patients with chronic hepatitis C have viral genome in the serum.[17,18]

Hepatitis D Virus Serology

Hepatitis delta virus (HDV) is a small RNA virus that causes hepatitis in those who are carriers of HBV (superinfection) or can be transmitted simultaneously along with HBV (coinfection). Both forms of HDV infection should be considered separately because of the differences in the prognosis and the serological changes (Figs. 4 & 5). Igm anti-HDV appears earlier

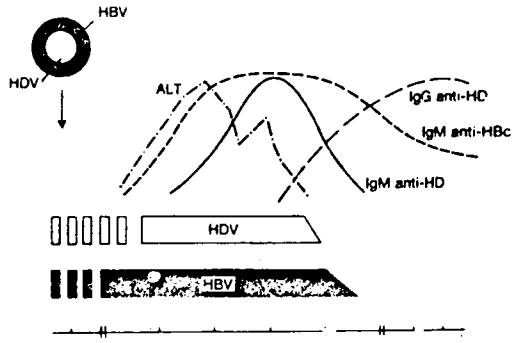

Fig. 3. Hepatitis "D" virus & Hepatitis B virus coinfection.

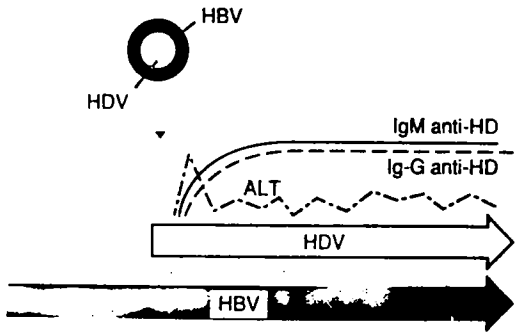

Fig. 4. Hepatitis D virus super infection.

during the course of HDV infection and is more reliable as a single diagnostic test for infection with HDV. Igm anti-HDV continues to persist in patients with chronic delta hepatitis while in acute resolving infection its levels disappear over few months. Anti-HDV appears late during HDV infection and infrequently anti-HDV may not be detectable. majority of the patients with co-infection (HDV & HBV) clear HBV and HDV whereas the patients with superinfection develop chronic rapidly progressive delta hepatitis.[19]

HDV antigen tests have been developed which are presently being used for re-

search and are not applicable for clinical use.[18] HDV RNA is being detected by molecular hybridization or by PCR and is used for monitoring therapy. Diagnosis of HDV can be established in the presence of clinical features of acute hepatitis and positivity of HBsAg & anti-HDV in the serum. Coinfection can be diagnosed when the serum is positive for Igm anti-HBC along with anti-HDV. HDV infection should be considered as superinfection when serum is positive for HBsAg anti-HDV and is negative for Igm anti-HBc. Chronic HDV can be diagnosed with clinical features of chronic hepatitis and persistence of HBsAg and anti-HDV in high titers in serum for 6 months or more. Diagnosis of HDV infection can be confirmed by the presence of HDV RNA in the serum or liver.[19]

Prevalence of Hepatitis B, C and D amongst Multitransfused Thalassemics

Majority of thalassemic children in this country despite repeated transfusion have not been investigated for the presence of all identificable hepatotropic viral markers. Seven different serological patterns were observed in India while evaluating the prevalence of hepatitis B.[20] Administration of hepatitis B vaccination in these children without assessing their pre-vaccination HBV exposure status may complicate the matter further if all the serological tests for HBV are not performed. Vaccinated children will develop anti-HBs but will not develop anti-HBC. Despite these limitations, the available data suggest the prevalence of hepatitis B, C & D is quite high amongst the thalassemic children (Table 1 & 2). Prevalence of HBsAg in thalassemic children is infrequent in the developed countries (0-9.6%) in contrast to similar prevalence amongst the thalassemics in India (7.5 to 59.6%). Such a low prevalence of HBsAg in the developed nations has been achieved by stringent screening of donors using sensitive techniques and administration of hepatitis B vaccine at the time of diagnosis of thalassemia. In India, the reason for such as high prevalence of HBV infection amongst the multitransfused thalassemic presumably is due to three obvious factors viz; (i) lack of awareness of HBV infection and its consequences amongst the thalassemics and Pediatricians; (ii) administration of HBV vaccine is not a routine at the time of diagnosis of thalassemia because of poor availability and (iii) high cost and unsatisfactory donor screening despite the recommendations of the government on the implementation of above recommendations will not only prevent HBV but also prevent HDV infection amongst these children.

Prevalence of hepatitis C infection has been increasing over the last several years and its prevalence amongst the multitransfused individuals is much higher in our country than the one reported from the developed countries (Table 1 & 2). In the absence of hepatitis C vaccine the routine screening of all the donors is the sole option to contain the spread of HCV infection. Anti-HCV positivity amongst the thalassemic children in Delhi city has been reported to vary from 32 to 62.2%.[37] Both HBV and HCV are established agents to cause cirrhosis of liver and hepatocellular cancer over a period of 15 to 20 years. Unless infection of such agents amongst the thalassemics is contained, they are likely to succumb from progressive irreversible liver damage during their adulthood, thus defying the basic aim of treating them with multiple transfusions.

TABLE 1. Prevalence of Hepatitis B, C, D in Multitransfused Indian Thalassemic Chidren

			Positivy in Percentage						
Study	Place	NO	HBsAg	Anti-HBsAg	Anti-HBc	Others	Over-All	Anti-HCV	Anti HDV
Mittal et al-88[21]	Delhi	27	14.8	48.1	51.8	29.6	74.1	–	–
Kapil D-89[22]	Delhi	50	10.0	40.0	62.0	–	80.0	–	–
Bhattacharya et al-92[23]	Calcutta	70	–	–	–	–	–	14.3	–
Gulati* et al-92[20]	Chandigarh	100	6.0	73.0	49.0	–	76	17.5	16.7
Amarapukar et al-92[24]	Bombay	40	45	–	–	–	–	7.0	3
William et al-92[25]	Delhi	54	7.4	–	59.2	–	66.6%	11.1	–
Jolly et al-92[26]	Lucknow	251	15.1	–	–	–	–	–	–
Chopra & Popli 95[27]	Delhi	37	59.6	–	–	21.6	–	62.2	–
Manglani et al-94[25]	Bombay	25	8.0	84.0	–	88.0	–	–	–
Choudhry et a-95[29]	Delhi	102	35.2	29.4	1.0	–	65.7	30.3	–

*Anti HBsAg positivity of 44% was observed in donor's blood.

TABLE 2. Prevalence of Hepatitis B & C in Thalassemia Major in the Developed Countries

			Positivity in Precentage				
Authors	Country	n	HBsAg	Anti-HBs + Anti-HBc	Anti-HBC	Overall Prevalance	Anti-HCV
Wonke et al-1990[30]	U.K.	73	0	27.04	–	27.4	23.2
de Montalembert et al 1992[31]	Belgium	15	0.0	73.3	26.7	–	13.3
	France	172	5.9	76.2	23.3	–	25
	Italy	118	1.2	57.6	49.2	–	50
Cacopardo et al 1992[32]	Italy	152	8	55	–	–	47
Dentico et al 1992[33]	Italy	114	9.6	29.8	9.1	47.4	–
Resti et al 1992[34]	Italy	78	–	–	–	–	83.3
alFawaz L & Ramia 1993[35]	S. Arabia	20	0	26.7	0	26.7	70.0
Lau et al 1993[36]	China	99	–	–	–	–	34.4

Evidence for Child to Child Transmission

There are evidences that hepatitis may be transmitted from child to child. Franks & his colleagues[38] have reported that 55% of 31 children born to HBsAg positive carrier had evidence of HBV infection, whereas 6.6% of 226 children born to HBsAg negative mothers had evidence of HBV infection. In this study authors had shown that in nearly half of the cases with chronic HBV infection in Asian children, it was not attributed to vertical transmission. In another study of 429 children born to Hmong refugees, it was observed that HBV infection was detected in 30% of children born to mothers who were positive for HBsAg and 11% of children born to HBsAg negative mothers.[39] These observations suggest that horizontal transmission of HBV from child to child occurs frequently. However, these observations need to be confirmed among the siblings of thalassemic children. William and their colleagues,[25] observed that 28.9% parents of thalassemic children had evidence of HBV infection. It appears most likely that they had acquired HBV infection from their children.

Outcome of Hepatitis

An outcome of post-transfusion hepatitis varies and is dependent upon multiple factors such as age at which the exposure has occurred, host immunity, type of viral hepatitis or its mutants, hepatic iron content of the individual, risk of repeated exposures and nature of therapy. Acute hepatitis B commonly has a self-limiting course and the infection subsides over few weeks. In contrast acute hepatitis 'C' frequently leads to chronicity and spontaneous recovery is rare. Sufficient data on the natural course of hepatitis in thalassemics is not available. Thalassemic children have a high risk of developing chronic HBV infection due to three reasons : (a) multiple exposure to hepatitis B virus infection (b) compromised immune competence (c) exposure at younger age which is a known risk factor for chronicity. Progressive disease due to hepatitis 'B' is believed to occur with the persistence of viral replication, viral mutations, transient exacerbation of viral replication or reinfection. Some patients lose HBeAg but continue to have liver disease and are positive for HBV DNA.[40,42] In a long term follow up of 207 HBsAg positive patients with abnormal liver function tests over 3.3 years, 27 deaths were observed and of these 16 (59%) died of liver failure, variceal bleeding or sepsis and 8 (30%) developed thepatocellular carcinoma.

Wonke & her colleagues[30] observed that the thalassemic children positive for anti-HCV had significantly higher plasma aspartate aminotransferase activity (mean 70.3 ± 10.2) and serum ferritin levels 4067 ± 2708 ng/ml) than the anti-HCV seronegative patients (AST 39.9 ± 15.6 U/L, serum ferritin 205 ± 2092 mg/ml). Thirty-six children underwent liver biopsy. Ten of the twenty-one children, positive for anti-HCV had histological evidence of chronic hepatitis or cirrhosis or both in contrast to 1 of 15 children who were seronegative for anti-HCV and had evidence of chronic viral hepatitis. Resti *et al*[37] in a study of 78 thalassemic children over 13 years, observed chronic hepatitis in 56.7% of cases following acute HCV infection but the persistence of anti-HCV antibodies did not correlate with the chronic evolution of liver disease. In contrast Lai &

his colleagues[43] observed two episodes of HCV viremia in two children due to reinfection with a different HCV strain and reactivation of primary infection in the third case. Their studies have clearly suggested the persistence and reactivation of HCV and even episodes of reinfection with different strains of HCV in these children. In a large multicentric transfusion transmitted viral study, the prevalence of an overall NANB hepatitis was 10.9% with a range of 5.4 to 15.3% of cases.[44] Several studies have indicated that nearly 50% of such patients develop histological evidence of chronic hepatitis.[45,48] Asymptomatic nature of the illness associated with fluctuation of ALT levels prevents the diagnosis of HCV infection. However, elevated levels of ALT reflect the ongoing viral replication. Anti-HCV persists in patients who progress to chronic hepatitis and it may disappear in those who recover clinically and biochemically. The findings of HCV RNA by PCR in patients who are seronegative for anti-HCV suggest that neither normalization of ALT nor disappearance of anti-HCV imply the complete recovery. Ten to fifteen percent of the patients make progress to cirrhosis and finally to liver cancer, 7 to 18 years after the onset of NANB hepatitis.[49,50] Seventy percent of Japanese patients with HCV infection had persistence of anti-HCV for prolonged periods.[51] Similar observations have been made by other workers.[52,53]

Our understanding of the natural course HCV infection is evolving. Factors which influence the rate of progression to cirrhosis include the age of exposure, duration of infection, degree of the damage at the initial biopsy[54] and recurrent exposures. In a study on post-transfusion hepatitis C, 20% of the patients had cirrhosis after 10 years, but 66 to 71% had bridging fibrosis or cirrhosis after 20 to 25 years. Nineteen percent of their cases progressed to hepatocellular carcinoma.[54] Seff & his colleagues[55] observed a small but significant increase in liver related mortality over 15 to 20 years.

Management

Multiple antiviral agents have been evaluated for the management of hepatitis B, & C (Table 3). In the initial studies adenine arabinoside were used but were withdrawn because of neuromuscular toxicity and limited efficacy.[56,57] Acyclovir of 6-dexoyacyclovir were found to be safe but the clinical trails revealed that they were not effective.[58] Clinical studies with other antiviral agents (Zidovudine, Ganciclovir) and immune modifiers (levamisole, steroids, interleukin 2) have revealed that these agents when used alone were ineffective or had unacceptable toxicity.[59] Among

TABLE 3. Drugs used for Chronic Viral Hepatitis

Antivirals
Interferons (alfa, beta, gamma)
Adenine Arabinoside (ara-A) and its derivatives
Acyclovir/Deoxyacyclovir
Ganciclover
zidovudine
Suramin
Ouinacrine
3-thiacytidine
Immuno modulators
—Corticosteroids (daily or pulses)
—Interleukin-2
—Thymosin.

the various agents evaluated for the management of hepatitis, interferon therapy has been found to be the most effective.[58] Currently the studies are in progress to evaluate the optimal dose and duration of therapy with interferons. Interferons in combination with other drugs are being tried to improve the results. Thymosin appears to be a promising antiviral agent. It is a hormone like polypeptide produced by the thymus epithelial cells. Its mechanism of action is possibly by the regulation of maturation of T cells which in turn modulates the interferon production and stimulates the expression of interleukins.[60] In a recent study Mutchnich and his colleagues[61] observed that 86% of the patients had a sustained loss of HBV DNA after six months of treatment with thymosin. Among other agents ribavirin which is a synthetic nucleoside with a broad antiviral property appears to be a promising agent.[62,63]

Interferon

With the advent of recombinant DNA technology three major forms of interferon (alfa, beta and gamma) have been made available. Alfa interferon is produced from monocytes, beta from fibroblasts and gamma from T lymphocytes. Alfa a& beta have more antiviral activity, but the majority of trials have employed alfa inteferon. More than 20 different subtypes of alfa interferon have been identified. [64] Use of recombinant interferon alfa-2b has been evaluated involving 162 patients in USA.[65] FDA has approved the use of interferon alfa-2b for hepatitis in 1992.[59]

Hepatitis B

Prevalence of hepatitis B in thalassemic children in the developing countries has lowered down (Table 2) and currently large number of non-thalassemic patients with hepatitis have been treated with interferon. Unfortunately interferon trials amongst thalassemic children with chronic HBV hepatitis are not available. Therefore, the overall results & the prognostic factors following interferon therapy amongst non-thalassemic chronic HBV infection are being presented.

It has been observed that 20 to 30% of patients with hepatitis B respond to interferon. However, in a well controlled multicentric study involving 169 patients, 37% of the patients lost HBeAg & HBV DNA, when patients were treated with interferon in a dose of 5 million units three times weekly for 16 weeks, while the HBeAg seroconversion in patients treated with daily 1 million units of interferon and untreated controls was significantly lower. There was also no difference in results between the patients treated with 5 million or 10 million units given three them weekly for 16 weeks.[66] serum aminotransferase levels returned to normal in 90% of the cases with a sustained loss of HBV DNA. Reactivation of infection was observed in 5 to 10% of cases within one year. HBsAg became negative in 10 to 15% of cases during therapy of within the first three months. Antibodies to HBsAg appear in 75 to 90% of such cases.[67] Long term histological studies over 2 to 9 years in HBsAg negative patients have demonstrated the absence of disease progression along with improvement in the histological changes. Over the years, several clinical features have been identified which help the clinicians to predict good response to therapy. These factors include (a) history of acute hepatitis (b) short duration of the disease

(c) female sex (d) high levels of serum aminotransferase at the onset of hepatitis (e) low serum levels of HBV DNA (< 100 pg/ml) (f) low levels of HBeAg and (g) absence of other medical problems involving other organs.

Hepatitis C

Large number of studies on interferon amongst post-transfusion chronic non-A, non-B hepatitis patients were initiated before the identification of HCV in 1989. On retrospective serological testing these patients were confirmed to have HCV infection. In one of the large trial of interferon amongst patients of chronic non A, non B hepatitis, normalization of serum ALT was observed in 38% of those treated with 3 Mu of interferon thrice weekly for 6 months, 16% treated with lesser dose and in 4% of untreated controls.[68] Significant reduction in labular and periporatal inflammation was observed in treated patients. Subsequently, 13 randomized controlled studies on interferon have been reviewed by Davis & Lau.[69] Interferon dose in three studies varied between 1-10 Mu thrice weekly for 1-18 months. Results in these studies were similar. Normalization of ALT was seen at the end of the study in 2.6% (range 0-6.6%) of the control group, 27% (range 16-45%) in those treated with 1 Mu of interferon for 6 months, in 33% when treated with 2 Mu for 6 months, and 41.5% (range 35-73%)in those treated with 3 Mu for 6 months.[69] Results of interferon therapy were also identical in the two European multicentric studies,[55,70] In all these studies HCV RNA became undetectable with the normalization of ALT during therapy.[71] Unfortunately majority of patients (50-80%), who had responded to treatment had relapsed within 6 months of cessation of interferon therapy.[69,70,72] Patients with relapse of MCV infection can be treated again with second course of interferon therapy.[73] Pre-treatment characteristics are useful in identifying patients who are likely to achieve remission. Patients with milk histological changes and low virus load are likely to respond.[74] However, such patients need not be treated as the progression of the disease is very slow in these cases.

Results of interferon therapy in small

Table 4. Results of Interferon Therapy in Thalassemics with Hepatitis C

Ref.	N	Dose of Interferon	Immediate response (%)	Follow up period months	Long term response (%)
Marco et al-1992[75]	12	5 Mu/M² TIW x 8 wk Followed by 3 Mu/M² TIW x 18 wk	77	12	55.5
Wonke et al-1993[76]	15	3 Mu/M² TIW 6M	53	–	–
Clemente et al 1994	51	3 Mu/M² TIW x 15 M	41	36	37

number of thalassemic children with hepatitis C have been tabulated in Table 4. In all these studies response criteria were normalization of ALT levels and loss of HCV RNA in the serum. An immediate response varied between 41 to 77 percent with long term response being 37 to 55.5 percent of cases.[75,77]

In conclusion we will like to emphasize the following facts :

(1) Prevalence of hepatitis B virus and hepatitis 'C' virus infection amongst multitranfused thalassemics in India is alarmingly high and aggressive strategies to control such infection are necessary to prevent the high morbidity and mortality rate amongst these children when they reach their youth.

(2) Stringent donor screening facilities, frequent cross checks of such screening facilities with quality control are essential to decrease the prevalence of these infections. Public awareness about these problem s through extensive media coverage along with the availability of cheaper indigenously prepared HBV vaccines is of importance for prevention of HBV infection. Free availability of kits for testing anti-HCV, along with the availability of trained personnel are not so difficult measures to control the phepatotropic viral infections which have impact on high morbidity and mortality.

(3) Interferon trials either alone or in combination with other antiviral agents amongst chronic HBV & HCV infection in multitransfused thalassemics in this country are required on priority to evolve a therapeutic policy for these children.

References

1. Choudhry VP, Desai N, Pati HP and Nanu A. Current management of homozygous beta thalassemia. *Ind. Pediatr* 1991; 28 : 1221-1229.
2. Froesner GG, Schomerus H, Widemann KH, et al. Diagnostic significance of quantitative determination of hepatitis B surface antigen in acute and chronic hepatitis B infection. *Eur J Clin Microbiol* 1982; 1 : 52-58.
3. Hoofnagle JH, Shafritz DH and Popper H. Chronic type B hepatitis and the "healthy" HBsAg carrier state. *Hepatology* 1987; 7 : 758-763.
4. Ou J, Laub O and Rutter W. Hepatitis B vbirus gene function. The precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. *Proc Antl Acad Sci USA* 1986; 83 : 1575-1582.
5. You JY, Howerd R, Waggoner JG and Hofnagle JH. Peroxidase anti-peroxidase detection of hepatitis B surface and core antigen in liver biopsy specimens from pateints with chornic type B hepatitis. *J Med Virol* 1978; 2 : 77-87.
6. Hadziyannis SJ, Lieberman HM, Karvountzis GG and Shafrtiz DA. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg vs anti-HBe positive carriers of hepatitis B virus. *Hepatology* 1983; 3 : 656-662.
7. Kenako S, Miller RH, di bisceglie AM, et al. Detection of hepatitis B virus DNA in serum by polymerase chain reaction. *Gastroenterology* 1990; 99 : 799-804.
8. Perrillo RP, Schiff ER, Davis GL, et al. A randomized controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N Engl J Med* 1990; 323 : 295-301.
9. Scolnick EM, McLean AA, West DJ, et al. Serologic resoponses in healthy adults of a hepatitis B vaccine made by recombinant DNA. *JAMA* 1984; 251 : 2812-2815.
10. Shiels MT, Taswell HF, Czaja AJ, et al. Frequency and signficance of concurrent

hepatitis B surface antigen and antibody in acute and chornic haptitis B. *Gastroenterology* 1976; 93 : 675-680.
11. Chau KH, Hargio MP, Decker RH, et al. Serodiagnosis of recent hepatitis B infection by Igm class anti-HBc. *Hepatology* 1983; 3 : 142-149.
12. Sjogren M and Hoofnagle JH. Immunoglobulin M antibody to hepatitis B core antigen in patients with chronic type B hepatitis. *Gastroenterology* 1985; 89 : 252-258.
13. Carman WF, Jacyna MR, Hadziyannis S, et al. Mutation preventing formation of hepatitis Be antigen in patients with chronic hepatitis B infection. *Lancet* 1989; 335 : 588-591.
14. Choo Q-L,Luo G, Winer AJ, et al. Isolatio of aa DNA Clone dervied from a blood-borne non-A, non-B viral hepatits genome. *Science* 1989; 244 : 359-362.
15. Kuo G, Choo Q-L, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non B hepatitis. *Sceince* 1989; 244 : 362-364.
16. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989; 321 : 1494-1500.
17. Garons JA, Tedder RS, Briggs M, et al. Detection of hepatitis C viral sequences in blood donation by "nested" polymerase chain reaction and prediction of infectivity. *Lancet* 1990; 336 : 1419-1422.
18. Hoofnagle JH and Bisceglie AMD. Serologic diagnosis of acute and chronic viral hepatitis, *Seminar Liv Dis* 1991; 11 : 73-83.
19. Smedile A, Lavarini C, Vrivelli O, et al. Radioimmuno assay detection of Igm antibodies to the HDV-associated delta antigen. Clinical significance in delta infection. *J Med Virol* 1982; 9 : 131-138.
20. Gulati S, Marwala RK, Dilawari JB, et al. Serologial responses to hepatitis B virus infection in multitransfused thalassemic children. *Ind pediatr* 1991; 29 : 73-77.
21. Mittal MK, Vij JC, Talukdar B, Sachdev HPS and Saini L. Prevalence of hepatitis B virus markers in multitransfused thalassemic patients. *2nd Pediatr* 1988; 25 : 161-165
22. Kapil D, Growth and Development, liver and cardiac dysfunction following multiple blood transfusion in patients of thalassemia. Thesis submitted to All India Institute of Medical Sceinces. New Delhi 1989.
23. Bhattacharya DK, Bhattacharjee S, De M, and Lahiri P. Prevalence of hepatitis C in transfusion dependent thalassemics and haemophilics. *Ind J Med Ped* 1991; 94 : 430-432.
24. Amarapukar DN, Kumra A, Vaidya S, et al. Frequency of hepatitis B, C and D and human immunodeficiency virus infection in multitransfused thalassemics. *Ind J Gastroenterology* 1992; 11 : 80-1.
25. William TN, Wonke B and Donoku SM, A Study of Hepatitis B and C prevalence and liver unction in multitransfused thalassemia and their parents. *Ind Peiatr* 1992 ; 29 : 1119-24.
26. Jolly JG, Agnihotri SK, Choudhry N, and Gupta D, Evaluation of haemotherapy in thalassemia (20 years of India experience). *J Ind Med Anoc* 1992; 90 : 7-9.
27. Chopra K and Popli V. Prevalence of hepatitis B and hepatitis C in mulitiransfused thalassemics. XXXII National confernce of India Academy of Pediatrics (Abstract) 1994; 44-45.
28. Manglani M, Kanabia S, Bhatia N, et al. Liver function tests, hepatitis-B markers and HIV in multiply transfused thalassemics XXXII Natinal conference of Indian Academy of Pediatrics (Abstract) 1994 : 53-54.
29. Choudhry UP, Charya SK, Prasanna U, et al. Hepatitis B and C viral markers amongst multitransfused thalassemic children (paper submitted for publication).
30. wonke B, Hoffbrand A.V. Brown D, et al.

Antibody to hepatitis C virus in multiply transfused patients with thalassemia major. *J clin Path* 1990; 43 :638-640.

31. de Montaiembert M, Costagliola DG, Lefrere JJ, et al. Prevalence of markers for human immunodeficiency virus types I and II human Tlymphotropic virus type I, cytomegalovirus, and hepatitis B and C virus in multiply transfused thalassemia patients. *Transfusion* 1992; 32 : 509-512.
32. Ca copardo B, Runo R, Fatuzzo O, et al. HCV and HBV infection amoung multitransfused thalassemics from eastern sicily. *Infection* 1992; 20 : 83-85.
33. Dentico P, Buongiorno R, Volpe A, et al. Long term persistence of anti HBs after hepatitis B immunization in thalassemic patients. *Infection* 1992; 20 : 276-278.
34. Resti M, Azzari C, Rossi Me, et al. Hepatitis virus antibodies in a long term follow up of beta thalassemic children with acute and chronic non-A non-B hepatitis. *Eur J Pediatr* 1992; 1151 : 573-576.
35. al-Fawaz I and Ramia S. Decline in hepatitis B infection in sickle cell anemia & beta thalassemia major. *Arch Dis child* 1993; 69 : 594-596.
36. Lau YL, Chow CB, Lee AC, et al. Hepatitis C virus antibody in multiply transfused chinese with thalasemia major *Bone Marrow Transplant* 1993; 12 : 26-28.
37. Resti M, Azzasi C, Rossi ME, et al. Hepatitis C virus antibodies in a long term follow up of beta-thalassemic children with acute and chronic non-A and non-B hepatitis. *Eur J Pediatr* 1992; 151 : 573-760.
38. Franks AL, Berg CJ, Kane MA, et al. Hepatitis B virus infection among children born in the United States to southeast Asian refugees. *N Engl J Med.* 1989; 32 : 1301-1305.
39. Hurie MB, Mast EE, Davis JP. Horizontal transmission of hepatitis B virus infectrion to United States - born children of Hmong refugees. *Pediatrics* 1992; 89 : 269-273.
40. Carman WF, Jacyna MR, Hadziyannis S, et al. Mutation preventing formation of hepatitis Bc antigen in patients with chronic hepatitis B infectin. *Lancet* 1989; 2 : 588-591.
41. Brunetto MR, Stempler M, Borino F, et al. A new haptitis B virus strain in patients with severe anti HBe positive chronic hepatitis B *J Hepatol* 1990; 10 : 258-261.
42. Tong MJ, Sampliner RE, Govindrajan S, et al. Spontaneous reactivation of hepatitis B in chinese patients with HBsAg positive chronic active hepatitis. *Hepatology* 1987; 7 : 713-718.
43. Lai ME, Mazzoleni AP, Srgiolu F, et al. Hepatitis C virus in multiple episodes of acute hepatitis in polytransfused thalassemic children. *Lancet* 1994; 343 : 388-90.
44. Aach RD, Lander JI, Sherman LA, et al. Transfusion transmitted viruses. Interim analysis of hepatitis among transfused and non-transfused patients. In : Vyas GN, Cohen SN. Schmied R, Eds. *Viral hepatitis*. Philadelphia, Franklin Institute Press, 1978 : 383-396.
45. Dienstage JL : non-A, Non-B hepatitis I. Recognition, epidemiology, and clinical features. *Gastroenterology* 1983; 85 : 439-462.
46. Dienstag JL. non-A, non-B hepatitis. II. Experimental transmission putative virus agents and markers, and prevention. *Gastroenterology* 1983; 85 : 743-768.
47. Bradley DW, Maynard JE. Etiology and natural history of post-transufion non-A, non-B hepatitis. *Semin Liver Dis* 1986; 6 : 56-66.
48. Alter HJ : Chronic sequences of non-A, non-B hepatitis. In : Seef LB, Lewis JH, eds. *Current perspectives in Hepatology*. New York, Plenum Publishing. 1989 : 83-97.
49. Kiyosawa K, Akahoma Y, Nagata A, Furuta S. Hepatocellular carcinoma after non-A, non-B postransfusion hepatitis. *Am J Gastroenterol* 1987; 78 : 777-781.
50. Tremolada F, Benvegun L, Casarin C, et

al. Antibody to hepatitis C virus in hepatocellular carcinoma. *Lancet* 1990; 1 : 300-301.
51. Nishioka K : Postransfusion hepatitis C. Proceedings of the second internatinoal symposium on HCV Los Angeles. November 1990. p 36.
52. Bruix J, Calvet X, Costa J, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; 2 : 1004-1006.
53. Colombo M, Choo GL, Del Ninno E. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; 2 : 1006-1003.
54. Tabashashi M, Yamada A, Mijamoto R, et al. Natural course of hepatitis C. *Am J Gastroenterol* 1993; 88 : 240-243.
55. Seeff LB, Bushell-Bales 2, Wrigth E, et al. Long term mortality after transfusion associated non-A, non-B hepatitis. *N Engl J Med* 1992; 327 : 1906-1911.
56. Hoofnagle JH, Hanson RG, Minuk GY, et al. Randomized, controlled trial of adenine arabinoside monophosphate for chronic type B hepatitis. *Gastroenterology* 1984; 86 : 150-157.
57. Perillo RP, Regenstein FG, Bodicky CJ, et al. Comparative efficacy of adenine arabinoside 5 monophosphate and prednisone withdrawal followed by adenine arabinoside 5' monophosphate in the treatment of chronic active hepatitis type B. *Gastroenterology* 1985; 88 : 780-786.
58. Alexander GJM, Fagan EA, Hegarty JE, et al. Controlled clinical trial of acyclovir in chronic hepatitis B virus infection. *J Med Viron* 1987; 21 : 81-87.
59. Perrillow RP, and Mason AL. Therapy for hepatitis B virus infection. *Gastro clin N Am* 1994; 23 : 581-601.
60. Sztein MB and Goldstein AL. Thymic Hormones a clinical update. *Springer Semin Immunopathol* 1986; 9 : 1-18.
61. Mutchinck MG, Appelman HD, Chung HT, et al. Thymosin treatment of chornic hepatitis B. A placebo controlled pilot trrial. *Hepatology* 1991; 14 : 409-415.
62. Jain S, Thomas HC, Oxford JS, et al. Trial of rivavirin for the treatment of HBsAg positive chronic liver disease. *J Antimicrob Chemiother* 1978; 4 : 367-373.
63. Fried MW, Fong TL, Swain MG, et al. Hepatitis B virus (HBV) DNA suppression during long-term therapy with ribavirin (abstract). *Gastroenterology* 1992; 102-A808.
64. Peters M. Mechanisms of action of interferons. *Semin Liver Dis* 1989; 9 : 235-239.
65. perriollo RP, Schiff ER, Davis GL, et al. A Randomized controlled trial of interferon alfa-2b alone and after prednisone withdrawal, in the treatment of chronic hepatitis B. *N Engl J Med* 1990; 323 : 295-301.
66. di Bisceglie AM, Bergasa NV, Fong TL, et al. A randomized controlled trial of recombinant alfa interferon therapy for chronic hepatitis B (abstract) *Hepatology* 1991; 13 : 70 A.
67. Karayiannis P, Kanatakis S, Thomas HC, Anti-HBs response in seroconverting chronic HBV carriers following alfa-interferon treatment. *J Hepatol* 1990; 10 : 350-352.
68. Davis GL, Balart LA, Schift ER, et al. Treatment of chornic hepatitis C with recombinant interferon alfa; A multicenter randomized, controlled trial. *N Engl J Med* 1989; 321 : 1501-1506.
69. Davis GL and Lau JYN. Hepatitis C. In : Haubrich W, Schaffner F, Berk J eds. *Bockus Gastroenterology*, Philadelphia: WB Sacenters, 1994.
70. Mar cellin P, Boyer N, Giostra E, et al. Recombinant human alfa interferon in patients with chronic non-A, non-B hepatitis. A multicenter randomized controlled trial from France, *Hepatology* 1991; 13 : 393-397.
71. Shindo M, Di bisceglie AM, Cheung L, et al. Decrease in serum hepatitis C virual RNA during alfa-interferon therapy for

chronic hepatitis C. *Ann inter Med* 1991; 115 : 700-704.
72. Tine F, Nagrin S, Crasci, A, et al. Interferon for non A non B chronic hepatitis. A metanlaysis of randomised clinical trials. *J Hepatol* 1991; 13 : 192-199.
73. Tolini G, Furlan C, Glimalid, F et al. One course versus two courses of recombinant of alfa interferon in chronic hepatitis C Arch. *Viral suppl.* 1992; 4 : 294-298.
74. Davis GL, Lindsay K, Albrecht J, et al. Predictors of response to recombinant alfa interferon treatment in patients with chronic hepatits C (abstract) *Hepatology* 1990; 12 : 905.
75. Di Marco U, Lo Iaconod, Capra M, et al. Alpha interferon treatment of chornic hepatitis C in beta thalassemia. *Gut* 1993; 34 : S 142-143.
76. Wonke B, Donohue SM, Haffbrand AV, et al. Recombinant alfa 2 B interferon (IFN) in the treatment of chronic hepatitis C disease in the thalassemia major. *Bone Marrow Transplant* 1993; 1 : 24-25.
77. Clemente MG, Congia M Lai ME, et al. Effect of iron. Overload on the response to recombinant interferon alfa treatment in transfusion dependent patients with thalassemia major and chronic hepatitis C. *J Pediatr* 1994; 125 : 123-128.

A BETTER MEASLES VACCINE

Despite the high coverage with current vaccines, measles continues to exact a tremendous toll on the lives of small children. Even with global vaccine coverage running of around 78%, nearly 1.5 million children continue to die from the disease each year.

At present, measles immunization in industrialized countries is given at the age of 12-15 months, so that the early immunity acquired from the mother does not interfere with the action of the vaccine. Unfortunately, in developing countries where measles transmission can be very intense and the risk of infection is generally greater than in developed countries, the awning of maternal-acquired immunity can expose children to infection long before their first birthday. WHO has therefore recommended that measles vaccinations in developing countries should be administered at nine months.

A measles vaccine that could be effective between the ages of two and six months would be most valuable. It would fill the "gap" that often occurs after the loss of maternal-acquired immunity, and would enable this vaccine to be include in the existing schedules of the Expanded programme on Immunization.

An ideal measles vaccine should be safe, and should induce lifelong protective immunity in almost 100% of recipients with a single dose administered shortly after birth. It should also be compatible with other antigens dispensed at the same time, provoke mucosal immunity, interrupt wild measles transmission, retain its potency even at 45°C for seven days, and not be much more expensive than the current vaccine.

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