Decline in hepatitis B infection in sickle cell anaemia and β thalassaemia major

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Abstract

Seventy five Saudi children, 55 with sickle cell anaemia and 20 with β thalassaemia major, who were negative for all hepatitis B virus (HBV) markers five years ago were recently investigated for exposure to HBV and hepatitis C virus (HCV) infection. Of the 55 patients with sickle cell anaemia and 20 with β thalassaemia major, 20 and five patients respectively had been vaccinated against HBV earlier and all of them still had protective antibody (anti-HBs 42-96 IU) 3-5 years after vaccination and there was no vaccine failure. Among the non-vaccinated children the exposure rates to HBV were 14.3% among those with sickle cell anaemia and 26.7% among those with β thalassaemia and this was not statistically significant when compared with the exposure rate to HBV among the general paediatric population (20.1%). Anti-HCV positivity among those with β thalassaemia major and sickle cell anaemia was 70% and 18.2%, respectively, and this was significantly higher than anti-HCV positivity among the control group (0.8%). Anti-HCV positivity was directly related to the amount of blood transfused and to the duration of transfusion. The results of the study show that although the exposure rates to HBV among patients with sickle cell anaemia and β thalassaemia major were not significantly different than that among the general paediatric population, infection with HBV still takes place among non-vaccinated patients despite strict precautionary measures taken. Hence early vaccination against HBV would probably be the only effective way of controlling HBV infection. For HCV infection, and because a vaccine against HCV is still not available, preventive measures such as blood screening for anti-HCV before transfusion and stringent infection control measures are crucial steps to be implemented for the control of spread of HCV among these groups of patients.

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Hepatitis C virus (HCV)¹ was recently identified as the major causative agent of transfusion non-A, non-B hepatitis^{2,3} and diagnosis of infection has been made possible by the development of an anti-HCV assay.⁴ Groups at high risk of infection with HCV are those who receive multiple blood transfusions such as haemophiliacs,⁵ patients on haemodialysis,^{6,7} and those with thalassaemia.⁸

In addition to HCV, multitransfused patients are also at a high risk of acquiring hepatitis B virus (HBV) infection.^{9,10} Previously we have shown that multitransfused Saudi patients with sickle cell anaemia and thalassaemia have a high exposure rate to HBV.¹¹ Because of this, strict measures were implemented at our hospital to control the spread of HBV infection. These included improved infection control, vaccination against HBV, and using third generation techniques for screening for hepatitis B surface antigen (HBsAg). This report is concerned with the prevalence of HBV infection in the above mentioned two groups of Saudi patients five years after implementation of the control measures and also with the prevalence of anti-HCV using the recently available screening and confirmatory second generation enzyme immunoassay techniques.

Patients and methods

PATIENTS STUDIED

Fifty five Saudi children with sickle cell anaemia (30 boys, 25 girls; age range 2.5-14 years) and 20 Saudi children with β thalassaemia major (11 boys, nine girls; age range 1-14 years) who attended the haematology clinic at King Khalid University Hospital, Riyadh, were included in this study. The diagnosis was confirmed by haemoglobin electrophoresis on cellulose acetate at pH 8.6 and citrate agar at pH 6.0. Adult and fetal haemoglobins were estimated by the elution method and alkaline denaturation respectively as described earlier.¹² Routine screening for HBsAg and antibody to HBsAg (anti-HBsAg) were done on each patient on the initial visit to the clinic. No screening for antibody to HCV (anti-HCV) was performed as the test was not available then. Among the 55 children with sickle cell anaemia and the 20 children with β thalassaemia major, 20 (36.4%) and five (25%), respectively, were already vaccinated (three doses) against HBV. Those who were vaccinated were the only ones whose parents accepted the theoretical risk from a plasma derived vaccine. Genetically derived HBV vaccine became available only recently at our hospital.

CONTROL GROUP

The control group consisted of 120 Saudi children (60 boys, 52 girls; age range 1-12 years) who were investigated in another research project regarding the age specific prevalence of HBV markers among Saudi

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Table 1 HBV markers and antibody to HCV (anti-HCV) in multitransfused Saudi patients with sickle cell anaemia and β thalassaemia major

	No (%) HBV markers positive				
	HBsAg	Anti-HBc alone	Anti-HBc and anti-HBs	Anti-HBs alone	Anti-HCV positive
Controls (n=120)	7 (5.8)	1 (0.8)	14 (11.7)	3 (2.5)	1 (0.8)
Sickle cell anaemia (n=55)	0	0	5 (14.3)*	20 (36.4)†	10 (18.2)
β Thalassaemia major (n=20)	0	0	4 (26.7)*	5 (25.0)†	14 (70.0)

*These patients had no history of vaccination against HBV.

†These patients were vaccinated (three doses) against HBV.

children. The sampling strategy of these children has already been detailed.¹³ The control group were not vaccinated against HBV.

SEROLOGY STUDIES

All sera were tested for HBV markers (HBsAg; total antibody to HBsAg (anti-HBs); total antibody to hepatitis B core antigen (HBcAg) (anti-HBc), and to hepatitis B e antigen (HBeAg)) by the enzyme linked immunosorbent assay (ELISA) from Organon Teknika NV, Belgium and for anti-HCV by ELISA from Abbott Laboratories, North Chicago, Illinois, USA. All anti-HCV positive samples were confirmed by the Abbott HCV enzyme immunoassay supplemental assay. Specimens from children who were vaccinated against HBV were tested by ELISA from Organon Teknika and an anti-HBs titre of ≥ 10 mIU was considered positive. The procedures followed in all assays were as stated by the manufacturer.

SERUM ALANINE AMINOTRANSFERASE

Alanine aminotransferase activities were considered abnormal if they were at least 1.5 times the normal value (0–56 IU).

STATISTICAL ANALYSIS

Fisher's exact test and χ^2 test were used in the comparison of proportions. Mean values were compared using Student's *t* test.

Results

Prevalence of HBV markers and anti-HCV among multitransfused Saudi patients with sickle cell anaemia and β thalassaemia major is shown in table 1. Seven (5.8%) of the controls were HBsAg positive and none of the patients was a carrier. Among the 35 patients with sickle cell anaemia and who were not vaccinated against HBV five (14.3%) were exposed

to HBV (anti-HBc and anti-HBs positive) compared with 25 patients (14 anti-HBc and anti-HBs positive, one anti-HBc positive, three anti-HBs positive, and seven HBsAg positive) among the control group; this was not statistically significant ($p=0.535$). Among the 15 patients with β thalassaemia major who were not vaccinated against HBV four (26.7%) had evidence of exposure to HBV (anti-HBc and anti-HBs), which also was not statistically significant compared to the exposure rate to HBV (20.8%) among the control group ($p=0.815$). There was no vaccine failure against HBV among the 25 patients vaccinated and the anti-HBs titre range was 42–96 IU 3–5 years after vaccination.

Anti-HCV positivity among patients with β thalassaemia major was 70% compared with 18.2% among patients with sickle cell anaemia and 0.8% among the control group (table 1). Anti-HCV was significantly higher in those with β thalassaemia major than in those with sickle cell anaemia ($p<7\times 10^{-5}$) and among the control group ($p<10^{-8}$). Furthermore, anti-HCV positivity among the patients with sickle cell anaemia was significantly higher than that among the control group ($p<4\times 10^{-5}$). Among patients with sickle cell anaemia as well as among those with β thalassaemia major anti-HCV positivity was directly related to the amount of blood transfused (table 2). In addition to the amount of blood transfused, the duration of transfusion, particularly among the thalassaemics, influenced the rate of anti-HCV positivity (table 2).

Discussion

Our earlier data from the Riyadh area showed an exposure rate to HBV of 30.8% in the general paediatric population compared with 45.3% and 79.4% in sickle cell anaemia and β thalassaemia major patients, respectively.¹¹ The results of this study show clearly that the exposure rate to HBV has been reduced drastically in the same groups of patients who were not vaccinated against HBV. This reduction is most probably due to the use of the most sensitive third generation techniques for screening HBsAg in all blood donors during the past five years in addition to the strict precautionary measures applied at our hospital against spread of HBV infection. However, although the exposure rate among our patients was not different from that among the general paediatric population, infection with HBV still takes place among unvaccinated patients with thalassaemia and sickle cell anaemia. This infection is most probably due to frequent exposure to blood in these patients but intrafamilial spread cannot be ruled out.¹⁴ In all of our exposed patients, the infection was subclinical. The finding of no vaccine failure against HBV among our 25 vaccinated patients underscores the importance of vaccinating all patients with thalassaemia and sickle cell anaemia who have not been exposed to HBV. Early vaccination of these patients would probably be the only effective way of controlling HBV infection as HBsAg free blood cannot completely safeguard against HBV

Table 2 Comparison of patients with sickle cell anaemia and β thalassaemia major who were positive or negative for anti-HCV

	Sickle cell anaemia			β Thalassaemia major		
	Positive (n=10)	Negative (n=45)	<i>p</i> Value	Positive (n=14)	Negative (n=5)	<i>p</i> Value
Mean age (years)	10.1	11.0	>0.05	9.2	4.0	<0.03
Amount of blood transfused/year (units)						
≤5	1	40	<2×10 ⁻⁶	0	4	<0.003
>6	9	5		14	2	

infection.^{15 16} Vaccination against HBV is now a part of the Expanded Programme of Immunisation in Saudi Arabia.¹⁷

In contrast to the situation with HBV our patients, and particularly those with β thalassaemia major, are highly exposed to HCV (70%). This is much higher than what has been reported earlier⁵ where the first generation enzyme immunoassay test for anti-HCV was used. Hence, earlier data on anti-HCV have to be interpreted with caution in the light of relative insensitivity^{18 19} and specificity^{20 21} of this first generation immunoassay. A similarly high exposure rate to HCV infection has recently been reported among Saudi patients on haemodialysis using a second generation immunoassay (S Huraib *et al.*, personal communication). Whether some of these second generation assay results are false positive still awaits the development of more reliable confirmatory tests. The finding that only 15% of our anti-HCV positive patients had raised alanine aminotransferase activities and none of them was anti-HBc positive indicates that these surrogate markers are not reliable for predicting HCV infection and is in agreement with our earlier observation²² and those of others.²³ Higher frequency hospital admission and hence blood transfusion in thalassaemics compared with patients with sickle cell anaemia may explain the difference in exposure rate to HCV found among the two groups.

In conclusion, it seems therefore that until a vaccine against HCV becomes available, preventive measures such as blood screening for anti-HCV before transfusion and stringent infection control measures are crucial for the control of spread of HCV among these categories of patients. This is particularly important as recent evidence indicates that HCV does not illicit long term immunity and reinfection with a homologous strain is possible.²⁴

- 1 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; 244: 359–62.
- 2 Hopf U, Moller B, Kuther D, *et al.* Long-term follow-up of post-transfusion and sporadic chronic hepatitis non-A, non-B and frequency of circulating antibodies to hepatitis C virus (HCV). *J Hepatol* 1990; 10: 69–76.

- 3 Wang JT, Wang TH, Liu JT, Shen JC, Sung JL, Chen DS. Hepatitis C virus in a prospective study of post-transfusion non-A, non-B hepatitis in Taiwan. *J Med Virol* 1990; 32: 83–6.
- 4 Kuo G, Choo QL, Alter HJ, *et al.* An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989; 244: 362–4.
- 5 Bahakim H, Bakir TMF, Arif M, Ramia S. Hepatitis C virus antibodies in high risk Saudi groups. *Vox Sang* 1991; 60: 162–4.
- 6 Oguchi H, Terashima M, Tokunaga S, *et al.* Prevalence of anti-HCV in patients on long-term hemodialysis. *Nippon Jinzo Gakkai Shi* 1990; 32: 313–7.
- 7 Zeldis JB, Depner TA, Kuramoto IK, Gish RG, Holland PV. The prevalence of hepatitis C virus antibodies among hemodialysis patients. *Ann Intern Med* 1990; 112: 958–60.
- 8 Wonke B, Hoffbrand AV, Brown D, Dusheiko G. Antibody to hepatitis C virus in multiple transfused patients with thalassaemia major. *J Clin Pathol* 1990; 43: 638–40.
- 9 Kaime WN. Hepatitis B surface antigen in Nigerian children with sickle cell anaemia. *J Trop Pediatr* 1983; 29: 55–8.
- 10 Miele-Vergani G, Vergani D, White Y, *et al.* Hepatitis B virus infection in thalassaemia major treated in London and Athens. *BMJ* 1984; 288: 1804–7.
- 11 El-Hazmi MAF, Ramia S. Frequencies of hepatitis B, delta and human immune deficiency virus markers in multi-transfused Saudi patients with thalassaemia and sickle cell disease. *J Trop Pediatr* 1989; 92: 1–5.
- 12 El-Hazmi MAF. Hemoglobin disorders: a pattern for thalassaemia and hemoglobinopathies in Arabia. *Acta Hematol (Basel)* 1982; 68: 43–51.
- 13 Al-Faleh FZ, Ayoola EA, Aruf M, *et al.* Seroepidemiology of hepatitis B virus infection in Saudi Arabian children: a baseline survey for mass vaccination against hepatitis B. *Infect* 1992; 24: 197–206.
- 14 Ramia S. Intrafamilial clustering of hepatitis B virus (HBV) infection: study of 10 Saudi families. *Ann Trop Med Parasitol* 1990; 84: 623–8.
- 15 Papaevangelou G, Roumeliotou-Karayannis A, Tassopoulos N, Stathopoulou P. Diagnostic value of anti-HBcIgM in high prevalence areas. *J Med Virol* 1984; 13: 393–9.
- 16 Ramia S, Bakir TMF, Hossain A, El-Hazmi MAF. HBsAg and anti-HBsAg detection. Results of comparative testing in HBV endemic area. *Saudi Medical Journal* 1987; 8: 510–3.
- 17 Al-Faleh FZ, Ayoola EA, Al-Jeffry M, *et al.* Integration of hepatitis B vaccine into the expanded programme of immunization: the Saudi Arabian experience. *Annals Saudi Medicine* 1993; 13: 231–6.
- 18 Weiner AJ, Kuo G, Bradley DE, *et al.* Detection of hepatitis C viral sequences in non-A, non-B hepatitis. *Lancet* 1990; 335: 1–3.
- 19 Okamoto H, Okada S, Sugiyama Y, *et al.* Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5'-non-coding region. *Jpn J Exp Med* 1990; 60: 215–22.
- 20 Pascual M, Perrin L, Giostra E, Schifferli JA. Hepatitis C virus in patients with cryoglobulinemia type II. *J Infect Dis* 1990; 160: 567–9.
- 21 Boudart D, Lucas JC, Muller JY, *et al.* False-positive hepatitis C virus antibody tests in paraproteinaemia. *Lancet* 1990; 336: 63.
- 22 Ayoola EA, Huraib S, Arif M, *et al.* Prevalence and significance of antibodies to hepatitis C virus among Saudi hemodialysis patients. *J Med Virol* 1991; 35: 155–9.
- 23 Willems M, Tong G, Moshage H, *et al.* Surrogate markers are not useful for identification of HCV carriers in chronic hemodialysis patients. *J Med Virol* 1991; 35: 303–6.
- 24 Farci P, Alter HJ, Govindarajan S, *et al.* Lack of protective immunity against reinfection with hepatitis C virus. *Science* 1992; 258: 135–40.