Risks of seroconversion of hepatitis B, hepatitis C and human immunodeficiency viruses in children with multitransfused thalassaemia major

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Objectives: To estimate the risks of seroconversion of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency viruses (HIV) in children with multitransfused thalassaemia at a thalassaemic clinic in Kuala Lumpur, Malaysia.

Methods: Seventy-two children (39 males, median age 11.3 years, 2.5th–97.5th centile: 1.4–19.2 years) with thalassaemia major were studied. The risks of seroconversion of HBV, HCV and HIV were estimated by comparing the seroprevalences of hepatitis B surface antigen (HBsAg), anti-HCV and anti-HIV between a defined starting point and an end point. The end point was the point when latest serological results were available while the starting point was when regular transfusion was commenced, or approximately 5 years before the end point when the duration of transfusion was longer.

Results: The median duration of the study was 49 months (range 8–69 months, total 2953 patient-months). There were 2505 transfusion episodes and 4154 units of blood transfused (0.88 transfusion episode/patient per month, 1.41 units of blood transfused/patient per month). There were three new seroconversions for anti-HCV but none for HBsAg and anti-HIV. The risk of seroconversion for HCV was one in 1384 units of blood transfused (95% CI: 4000–472). The seroprevalence rates at the starting and end points were: HBsAg (1%, 1%), anti-HCV (10%, 13%) and anti-HIV (0%, 0%), respectively.

Conclusions: The estimated risk of acquiring HCV infection in children receiving multiple blood transfusions in this study is surprisingly higher than the generally accepted estimated risk. Other routes of transmission may be important. A prospective, multicentre study to estimate such risks more precisely is needed.

Key words: risk; seroconversion; thalassaemia major.

Children with thalassaemia major who received multiple blood transfusions are at risk of acquiring transfusion-related viral infections.1,2 Hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency viruses (HIV) are important causes of transfusion-related viral infections.3–8 Regular screening of blood for HBV, HCV and HIV viral markers in donated blood have resulted in a significant reduction of chronic HBV and HCV infections and HIV infections in multitransfused thalassaemias.5–8 Nevertheless, there remains a small risk for recipients of blood transfusion in acquiring infection through blood transfusions. This can take place when seronegative donors donate blood during the infectious window period at the time of seroconversion.9–11 Most of the published work on the seroprevalences of transfusion-related viral infection focused mainly on the point prevalence of HBV, HCV and HIV infections in transfusion-dependent thalassaemias.1,3–8 It is important to provide an accurate estimation of the risks of acquiring transfusion-related viral infections when counselling the parents of newly diagnosed thalassaemias major. Thus, efforts have been made to estimate the risks of such transfusion-related viral infections accurately.9–11

A retrospective cohort study was conducted to document the risks of seroconversion of HBV, HCV and HIV among a group of children with transfusion-dependent thalassaemias requiring multiple blood transfusions, attending a thalassaemic clinic in Kuala Lumpur, over a 5-year period of time.

METHODS

In Malaysia, universal hepatitis B vaccination to all newborn infants was started in 1989. In the Transfusion Medicine Unit, University of Malaya Medical Centre (UMMC), Kuala Lumpur, routine screening of donated blood for hepatitis B surface antigen (HBsAg) was started in 1978, anti-HIV in 1987 and anti-HCV in 1995.

Children with multitransfused thalassaemia major, attending the thalassaemic clinic of the Department of Paediatrics, UMMC, were studied to estimate the risks of acquiring HBV, HCV and HIV infections through blood transfusions. They were included if the frequency of blood transfusion was more than twice a year. The exclusion criteria were: (i) incomplete blood transfusion record; (ii) had blood transfusions in other institutions beside UMMC; or (iii) underwent bone marrow transplantation when multiple transfusions of blood and blood products were given.

The review was carried out in the months of May and June 2003. The record of blood transfusions was extracted from the case notes of patients and was cross-checked with the records of the Transfusion Medicine Unit, UMMC. The number of transfusion episodes, number of units of blood transfused during each hospital visit between a defined starting point and an end point were noted. Risk of seroconversion for each virus was estimated when new seroconversions were noted between the starting and the end points. The starting point was defined as the time when the first transfusion was given. For those children who had regular transfusions for more than 5 years, the viral markers obtained 5 years before the end point was taken as the starting point. The latest available status of viral markers of these patients was noted and was defined as the end point of the study.

The time after infection to positive serology (window period) are different for these three viral infections: 2–6 weeks for

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HBV,12 2 weeks for HIV13 and 10–16 weeks for HCV.14 Thus, infection may take place during the window period immediately before the baseline tests were performed and can be potentially labelled as new seroconversion. To avoid this, viral markers obtained during a period of 6 months before the baseline tests (starting point) were also noted.

Laboratory methods

In UMMC, multitransfused thalassaemic children undergo regular screening of viral markers, including HBsAg, anti-HBs, anti-HCV and anti-HIV antibodies at half-yearly intervals. Screening of blood for viral markers was performed at the Hepatitis and Virology Laboratory of UMMC. All HBsAg, HBeAg, anti-HBs, anti-HCV and anti-HIV antibodies were screened by using microparticle enzyme immunoassay (Abbott Laboratories, Chicago, Illinois, USA). Those who were positive for HBsAg were also screened for the presence of HBeAg. Sera positive for anti-HCV were confirmed with recombinant immunoblot assay.

Family members of seropositive cases

As there are reports of intrafamilial spread of HCV infection,15,16 information on the anti-HCV status of members of the same household on any new seroconversion were obtained either from their medical records or by direct questioning.

Statistics

Student’s t-test was used for statistical analysis and was expressed in proportions and 95% CI where appropriate. P-value of <0.05 was considered to be significant.

RESULTS

Demography

The review was conducted during May and June 2003. Seventy-six children had regular blood transfusions at the thalassaemic clinic of UMMC and fulfilled the study criteria. Four children underwent bone marrow transplantation during the study period and were excluded from the analysis. The records of the remaining 72 children (39 males, 33 females; median age 11.3 years, 2.5th–97.5th centile: 1.4–19.2 years) were analysed. Of these, 51 children (71%) had β-thalassaemia major, 20 (28%) had double heterozygous β-thalassaemia/HbE, whereas one child had delta/β-thalassaemia. One 19-year-old boy died of cardiac failure due to haemosiderosis. He was included in the analysis with the latest available viral markers before his decease, which was taken as the end point.

The median duration between the starting and the end points of these 72 children was 49 months (range 8–69 months, total 2953 patient-months). Between the starting and end points there were a total of 2605 transfusion episodes, with 4154 units of blood transfused (0.88 transfusion episode/month and 1.41 units of blood transfused/patient per month).

Seroprevalence of the cohort

At the starting point, the seroprevalence for HBsAg was 1% (one positive case), 10% (seven positive cases) for anti-HCV and 0% for anti-HIV (Table 1). At the end point, there were no new positive cases for both HBsAg and anti-HIV. There were, however, three new seroconversions for anti-HCV during the study period. The seroprevalence for anti-HCV at the end point was 14%. The risks of acquiring HBsAg and anti-HIV during the study period were zero, whereas that for anti-HCV was one in 868 transfusion episodes (95% CI: 2564–296) and one in 1384 units of blood transfused (95% CI: 4000–472).

The only child who was positive for HBsAg was negative for HBeAg. Anti-HBs antibody status was known in 45 children. Of these, 25 (55%) had a level >10 mIU/L, which was deemed to be protective while the remaining 20 children had an antibody level of <10 mIU/L. Presence of HCV-RNA was determined in four of the 10 children who were positive for anti-HCV and two were found to be positive. No genotyping of the HCV-RNA were performed.

Anti-HCV seropositive children

The 10 children who were seropositive for anti-HCV at the end point of the study were significantly older than those who were seronegative (median ± SD: 13.4 ± 5.15 years vs 9.8 ± 9.9 years, P < 0.05). All the seven children who were seropositive at the starting point of the study started regular transfusions before 1995, before routine screening of the blood for anti-HCV was commenced in UMMC. The three new seroconversions were aged 7.6, 11.3 and 19 years, respectively. The 19-year-old patient denied being sexually active. All remained persistently seropositive at the end point of the study.

Family members of anti-HCV seropositive cases

The three new seroconversions for anti-HCV had among them nine family members staying within the same household (two parents and one other sibling for each child). Information on their anti-HCV status was available on six members from two families. All were negative for anti-HCV.

DISCUSSION

The greatest threat to the safety of the blood supply is the donation of blood by seronegative donors during the infectious window period when the donors are undergoing seroconversion. Such people usually represent new or incident infections. The most direct way of estimating the risk of acquiring transfusion-related infections is to study the rate of acquiring a specific infection prospectively in transfusion recipients.17,18 For individuals receiving single transfusion, estimation of risks using such a direct method may be impractical because an enormously large number of recipients are required for the risks to be accurately measured.19–21 An indirect way of estimating such risks, such as risks for donating infected blood in the window period, was necessary.22–24 In the USA, the risks of donating blood during an infectious window period were estimated to be one in 493 000 for

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<tr>
<td>HBsAg 1 (1.4) 1 (1.4)</td>
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Table 1 Seroprevalence rates of hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV) and anti-HIV in 72 children with multi-transfused thalassaemia major in Kuala Lumpur, Malaysia.
HCV-RNA in addition to anti-HCV is clearly necessary. Certain authors have advocated screening of donated blood. Donation of blood by seronegative donors is potential of acquiring HCV infection despite regular screening of the number of units of blood transfused. We have not been able to seroconversion in multitransfused thalassaemias is related to the accuracy estimation difficult. Many authors noted that risk for new sample and the small number of new seroconversions make accurate estimation difficult. Howev

The accuracy of estimations in this study is imperfect, however, as there remain many confounding factors. For instance, the reason for the surprisingly much higher risk of acquiring HCV infection through blood transfusions noted in this study as compared to the much lower risk of donating infected blood during the window period is not apparent. Other modes of acquiring HCV infection may be possible. The issue of spreading of HCV infection within the family in these children, for instance, has not been sufficiently addressed. The small size of the study sample and the small number of new seroconversions make accurate estimation difficult. Many authors noted that risk for new seroconversion in multitransfused thalassaemias is related to the number of units of blood transfused. We have not been able to establish such risk due to the small number of the patients in this study.

Nevertheless, the result of this study highlighted the potential of acquiring HCV infection despite regular screening of donated blood. Donation of blood by seronegative donors during the infectious window period when the donors are undergoing seroconversion is a potentially important way of transmitting infection.

New screening test for HCV to reduce the risk further is clearly necessary. Certain authors have advocated screening of HCV-RNA in addition to anti-HCV. The major drawback of this method is that the cost may be prohibitively high for routine use in a developing country such as Malaysia. Others have suggested the use of blood from repeat donors, rather than from new donors. However, in UMMC, Kuala Lumpur, blood from new donors is accepted if they are tested negative for all the major pathogens.

Most of the published figures for the seroprevalence of anti-HIV among multitransfused thalassaemia major were zero, less than 5% for HBsAg, but significantly higher for anti-HCV (Table 2):3–8 In this study, the prevalence of anti-HCV among children with multitransfused thalassaemia was 14%, significantly higher than 0.6% among healthy children or 1.5% among the general population in Malaysia. Seven of the 10 thalassaemic children who were seropositive for anti-HCV in this study started having regular blood transfusion before routine screening of blood for anti-HCV. It is likely that the HCV infection was acquired through blood transfusions.

As in other studies, the seroprevalence of HBsAg and anti-HIV among children with multitransfused thalassaemia in this study was much lower than that of anti-HCV.Universal immunisation of all newborn infants with hepatitis B vaccine in Malaysia since 1989 certainly helped to reduce the incidence of HBV infection. The risks of acquiring HBV and HIV infections through transfusion of screened blood were also very small. Continued vigilance, however, must be exercised to ensure that multitransfused children do not acquire transfusion-related viral infections through blood transfusions.

**REFERENCES**