PREVALENCE OF TRANSFUSION ASSOCIATED INFECTIONS IN MULTITRANSFUSED CHILDREN IN RELATION TO MANDATORY SCREENING OF HIV IN DONATED BLOOD

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Abstract. Any change in risk behavior related to acquisition of human immunodeficiency virus (HIV) infection is likely to reduce simultaneously the risk for other agents transmitted through identical routes. A study carried out in the city of Delhi, India on the load of transfusion associated infections among multitransfused (MT) children in relation to mandatory screening of HIV infection in donated blood indicated unchanged prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) infections among the group of MT children transfused after the implementation of mandatory screening of HIV infections in blood banks, *ie* post-implementation period (prevalence of HBV, HCV and HDV being 32.8%, 31.3% and 1.6% respectively) compared to a group of MT children transfused over a similar duration before the implementation of mandatory screening *ie* pre-implementation period (prevalence of HBV, HCV and HDV being 28.1%, 26.6% and 1.6% respectively). However, reduction could be recorded in the prevalence of IgM and IgG classes of antibodies to both CMV and HSV-2 infections among MT children receiving transfusion during the post-implementation period (prevalence of 3.1% and 37.1% for CMV IgM and CMV IgG respectively; prevalence of 3.1% and 25% for HSV-2 IgM and HSV-2 IgG, respectively) compared to the group of MT children transfused in the pre-implementation period (prevalence of 15.6% and 56.3% for CMV IgM and CMV IgG respectively; prevalence of 18.8% and 45.2% for HSV-2 IgM and HSV-2 IgG, respectively). These reductions were statistically significant (p values < 0.02 and < 0.05 for CMV IgM and CMV IgG; p values < 0.01 and < 0.02 for HSV-2 IgM and HSV-2 IgG respectively). These observations were in accordance with the recorded reduction in the prevalence of CMV and HSV-2 infections and unaltered prevalence of HBV, HCV and HDV infections in the group of donors donating blood during the post-implementation period compared to those donating in the pre-implementation period. Study of epidemiological risk factors among blood donors showed a change in behavior towards safer sex practice with only 13.0% of donors in the post-implementation period having history of sex with one or more female commercial sex workers during their donation periods compared to 41.5% of donors in the pre-implementation period having similar history ($p < 0.001$). However no change could be recorded in the proportion of donors donating at frequency higher than the permissible guidelines among the two groups. The present study points out nosocomial transmission as well as limitations in the existing guidelines for screening of infectious agents in blood banks as possible incriminating factors towards acquisition of hepatitis virus infections in blood donors as well as in MT children.

INTRODUCTION

Mandatory screening of donated blood for human immunodeficiency virus (HIV) infection is likely to reduce simultaneously the risk of acquiring other transfusion mediated infectious agents known to be associated with HIV due to identical routes of entry (surrogate markers) (Simon and Bankhurst, 1984; Yosida *et al*, 1987; Quinn *et al*, 1988). However, regardless of their association with HIV, these agents other than HIV may independently be acquired by blood donors with high risk behavior specially in a country with low prevalence of HIV infection (Yadav *et al*, 1993). Recognizing the adverse sequelae associated with these non HIV agents among transfusion recipients, most developed countries have expanded their guidelines for screening infectious agents in donated blood and blood products either by including some of the agents like hepatitis C virus (HCV), cytomegalovirus (CMV) or by incorporating addi-

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tional markers complementary to the existing one as in case of hepatitis B virus (HBV) (Centers for Disease Control, 1991; Noal *et al*, 1994; Mirovic *et al*, 1971).

It is likely that commensurate with the introduction of mandatory anti-HIV screening in blood banks, concern about acquired immunodeficiency syndrome (AIDS) would offer a collateral benefit in terms of reduction of high risk behaviors among blood donors towards acquisition of infectious agents other than HIV with corresponding reduction in the load of these agents in transfused individuals. This gain could be significant for a developing country like India which is dependent on remunerated blood donors with documented high risk behaviors for its national blood supply (Chattopadhya *et al*, 1991). Thus, while the success of HIV screening in blood banks directed towards prevention of transfusion mediated HIV infection has been clearly established (Menitove, 1989) there is a need to determine the extent of benefit obtained, if any, from such screening of HIV infection regarding other important transfusion mediated infectious agents (Mattson *et al*, 1988).

In India, mandatory screening of donated blood was introduced in March 1989 in major cities including Delhi (Anonymous, 1989). The present study was taken up to assess any change in the magnitude of problem due to transfusion associated agents other than HIV consequent to implementation of mandatory screening of HIV infection in blood banks in the city of Delhi by comparing their prevalence among two groups of multitransfused (MT) children with thalassemia major receiving transfusions over a comparable time during the pre implementation period (before March 1989) and post-implementation period (after March 1989) respectively. In addition, we have attempted to correlate the prevalence of these markers in two groups of MT children with the prevalence of the identical markers, as well as with the pattern of risk behaviors among two groups of blood donors donating during the corresponding periods of transfusions in the two groups of MT children.

MATERIALS AND METHODS

Following the introduction of mandatory screening of donated blood for HIV infection in March 1989 in the city of Delhi, India (Anonymous, 1989) blood samples were routinely collected during the first two weeks of March 1989 for HIV screening among MT children of thalassemia major attending various hospitals in Delhi as well as from the professional blood donors registered with three local commercial blood banks. Following screening of HIV infection at the AIDS Reference Laboratory, NICD, Delhi these sera were stored at -70°C for future use. In addition to HIV screening, information was collected from the MT children regarding age, sex, number (units) and type of blood (*ie* whole blood or fractions) and blood products transfused, vaccination status against HBV and any history of sexual exposure or attendance in sexually transmitted diseases (STD) clinics. The professional donors, in addition to HIV screening, were subjected to confidential interviews for the study of epidemiological profile, *viz* age, sex, marital status, frequency of donation per month, history of having sex with male or female commercial sex workers (CSWs), parenteral drug abuse, attendance at sexually transmitted diseases (STD) clinics, jaundice, hospitalization and blood transfusion (Chattopadhya *et al*, 1991). In order to reassess the risk of transfusion mediated HIV infections in MT children after a seven year period following the implementation of mandatory HIV screening in blood banks in the city of Delhi, blood samples were again collected in June 1996 from different groups (other than 1989 group) of MT thalassemic children (64 cases) and professional blood donors (200 cases) from the city of Delhi with a history of receiving transfusion or donating blood respectively only after June 1989, *ie* for a maximum period of 7 years (post implementation period). The three month period from March to May 1989 was excluded from the post implementation period to minimize the possibility of including any MT child or blood donor harboring infection in the window phase due to acquisition of the infectious agent in the pre-implementation peirod shortly before the introduction of mandatory HIV screening in March 1989 (Nathanson, 1996). Identical information collected from the MT children and blood donors of the 1989 group were also collected from the MT children and blood donors of the 1996 group.

Matched with the duration of study period for MT children and blood donors in the post implementation period, *ie* 7 years for both the groups, equal numbers of sera were randomly selected from

the stored specimens from MT children (64) and blood donors (200) of 1989 collection, the criteria for selection being history of transfusions and donations respectively up to a maximum period of 7 years preceedings March 1989.

To avoid the influence of HIV status as a confounding variable and considering the primary objective of our study to assess the change in the magnitude of transfusion mediated agents other than HIV following implementation of mandatory screening of HIV infection in blood banks, two sera among blood donors and one serum from an MT child that were positive for HIV-1 infection in the 1989 collection were excluded from the study. This procedure enabled us to match HIV status between the groups of MT children and blood donors in the pre-implementation and post-implementation period.

Sera from each of the two subgroups of MT children and blood donors were subjected to detection of hepatitis B surface antigen (HBsAg), antibodies to hepatitis B surface antigen (anti HBs), hepatitis B core antigen (anti HBc), hepatitis C virus (HCV), hepatitis D virus (HDV), antibodies of IgM and IgG classes to CMV (CMV IgM and CMV IgG) and herpes simplex virus type 2 (HSV-2 IgG and HSV-2 IgM) by indirect micro-ELISA technics using commercial kits as employed earlier (Chattopadhya *et al*, 1997). *Treponema pallidum* specific antibodies were detected by *Treponema pallidum* hemagglutination assay (TPHA) (Chattopadhya *et al*, 1997).

Statistical analysis

Chi square (X^2) test with Yates correction was carried out for comparing the prevalence of various transfusion mediated markers in the two subgroups of MT children receiving transfusions before and after the implementation of mandatory screening for HIV infection in blood banks as well as among the two groups of donors donating in the corresponding periods. Comparison of the epidemiological markers among the two groups of blood donors, selected as above was also carried out by the same method (Daniel, 1995).

RESULTS

In the present study, the two subgroups of children, transfused during the pre-implementation and post-implementation periods belonged to comparable age groups between 2 to 8 years without significant difference in mean number of units of blood transfused (27.6 ± 15.4 and 30.9 ± 15.9 in the two groups respectively; $p > 0.05$). None of the children in the two groups gave a history of administration of blood products. The prevalence of infections due to HBV (32.8%), HCV (31.3%) and HDV (1.6%) among MT children receiving transfusion during the post-implementation period showed no statistical difference in the prevalence of similar infections (28.1%, 26.6% and 1.6% for HBV, HCV and HDV infections respectively) among those receiving transfusion during the pre-implementation period ($p > 0.05$, Table 1). However, there was significant reduction in the prevalence of both IgM and IgG classes of antibodies to CMV and HSV-2 recorded among MT children of the post-implementation period compared to the MT children of the pre-implementation period, $p < 0.02$ for CMV IgM and < 0.05 for CMV IgG; $p < 0.01$ for HSV-2 IgM and < 0.02 for HSV-2 IgG). *Treponema pallidum* specific antibodies were not detectable in either of the two groups of MT children (Table 1).

Comparative study of the profile of risk behavior among the two groups of blood donors donating during the periods corresponding to the period of transfusions in MT children showed all the donors in both the groups to be male and unmarried. Donors of both the groups were between 20 to 35 years of age with median ages of 26 years and 28 years, respectively. There was a significant reduction ($p < 0.001$) in the proportion of donors having a history of sex with one of more female CSWs during their tenure of donation in the post-implementation period (13.0%) compared to those in the pre-implementation period having similar history (41.5%, Table 2). None of the donors in either group admitted having sex with any female CSW prior to the tenure of donations. History of homosexual or bisexual exposure was not recorded in any donor among the two groups. On the other hand the donation characteristics in terms of frequency of donation were of similar pattern between the two groups of donors of pre-implementation as well as in post-implementation periods. Nearly all the donors in either group gave a history of donating at least once in two months as opposed to the recommended guideline of a minimum of three months interval between two consecutive donations (Saran

Table 1

Prevalence of serological markers of transfusion associated infections in MT children in relation to mandatory screening of HIV infection in blood banks in the city of Delhi, India*.

Serological markers	Period of transfusion		p-value
	Pre-implementation period n = 64	Post-implementation period n = 64	
HBsAg	18 (28.1)	16 (25.0)	NS
Anti HBs	2 (3.1)	1 (1.6)	NS
Anti HBc	19 (29.7)	15 (23.4)	NS
At least one HBV marker	21 (32.8)	18 (28.1)	NS
Anti HCV	20 (31.3)	17 (26.6)	NS
Anti HDV	1 (1.6)	1 (1.6)	NS
CMV IgM	10 (15.6)	2 (3.1)	< 0.02
CMV IgG	36 (56.3)	24 (37.5)	< 0.05
HSV-2 IgM	12 (18.8)	2 (3.1)	< 0.01
HSV-2 IgG	29 (45.2)	16 (25.0)	< 0.02

* Before and after the implementation of mandatory screening of HIV infections. Figures within parenthesis indicate percentage of various markers.
NS = Not significant (p value > 0.05)
Note: In both the groups evidence of infection due to *T. pallidum* were not detectable by TPHA.

Table 2

Profile of epidemiological risk factors in blood donors in relation to mandatory screening of HIV infection in blood banks in the city of Delhi, India*.

Risk factors	Period of donation		p-value
	Pre-implementation period n = 200	Post-implementation period n = 200	
Donation frequency			
(a) ≥ once per two months	196 (98.0)	188 (94.0)	NS
(b) ≥ once per month**	117 (58.5)	130 (65.0)	NS
(c) ≥ twice per month***	41 (20.5)	47 (23.5)	NS
Sex with one or more CSWs during the duration of donation	83 (41.5)	26 (13.0)	< 0.001
History of visiting STD clinic	6 (3.0)	5 (2.5)	NS

* Before and after the implementation of mandatory screening of HIV infection in blood banks.
** Subgroup of (a)
*** Subgroup of (a) and (b)
Figures within parenthesis indicate percentage of various markers
NS = Not significant (p - value > 0.05)
Note: (i) None of the donors in the above group had history of homosexual exposure, parenteral drug abuse, jaundice, hospitalization or blood transfusion.

Table 3

Prevalence of transfusion associated infections in blood donors in relation to mandatory screening of HIV infection in blood banks in the city of Delhi, India*.

Serological markers	Period of donation		p-value
	Pre-implementation period n = 200	Post-implementation period n = 200	
HBsAg	14 (7.0)	10 (5.0)	NS
Anti HBs	37 (18.5)	30 (15.0)	NS
Anti HBc	16 (8)	11 (5.5)	NS
At least one HBV marker	53 (26.5)	44 (22.0)	NS
Anti HCV	9 (4.0)	8 (4.0)	NS
Anti HDV	3 (1.5)	2 (1.0)	NS
CMV IgM	19 (9.5)	4 (2.0)	0.001
CMV IgG	180 (90)	135 (67.5)	< 0.001
HSV-2 IgM	10 (5.0)	2 (1.0)	0.02
HSV-2 IgG	145 (72.5)	114 (57.0)	0.001
Antitreponemal antibody	8 (4.0)	6 (3.0)	NS

* Before and after the implementation of mandatory screening of HIV infections in blood banks.
Figures within parenthesis indicate percentage of various markers.
NS = Not significant (p-value > 0.05)

and Makroo, 1991). As many as 58.5% of the donors in the pre-implementation period had the practice of donating at least once per month, 20.5% of whom gave history of donating at the frequency of twice or more per month while 65% of donors donating in the post-implementation period gave a history of donation at least once per month, 23.5% of whom admitted to be donating at least twice per month (Table 2). Very few donors in both the groups (3.0% and 2.1%) had a history of visiting STD clinics ($p > 0.05$), while none of the donors in either group gave a history of parenteral drug abuse, clinical jaundice, blood transfusion or hospitalization (Table 2).

A decline in the prevalence of CMV and HSV-2 both in terms of IgM and IgG classes of antibodies was recorded in the group of donors donating in the post-implementation period compared to those donating in the pre-implementation period. However, no difference was recorded in the prevalence of various markers of HBV and as well as that of HCV infection in the two groups. Prevalence of *T. pallidum* infection was recorded as low in both groups of donors without any statistical difference ($p > 0.05$, Table 3).

DISCUSSION

There are a number of reports on the prevalence of HBV infection among MT children from India (Gulati *et al*, 1992; Patwari *et al*, 1986; Udani *et al*, 1986) although reports on other hepatitis viruses are scarce (William *et al*, 1992) while to the best of our knowledge there are hardly any studies on the magnitude of the problem in MT children in relation to other agents like CMV and HSV-2 except two of our earlier reports (Yadav *et al*, 1991; Chattopadhya *et al*, 1997).

The IgG class of antibodies evoked by any transfusion mediated infectious agent in a multitransfused individual is likely to be detectable for a longer period due to its stability and thus its detection at a given point of time could be the reflection of infection by the agent acquired any time over the previous years of transfusions (Weise, 1987). As a consequence, the assessment of efficacy of an inter-

vention measure adopted in the transfusion setting for any infectious agent based on the study of IgG class of antibodies among transfused individuals is likely to be difficult in the absence of information regarding the prevalence of IgG antibodies against the agent among the same transfused group in the pre-intervention period. Inclusion of two different groups of MT children transfused exclusively during the periods before and after the implementation of mandatory HIV screening in blood banks provided us the opportunity to obviate this limitation. Moreover, during the entire period of the present study, *ie* before and after the introduction of mandatory anti-HIV screening in blood banks in Delhi there has not been any change in the national guidelines for screening of infectious agents in donated blood, *viz* HBV (based on HBsAg alone), *T. pallidum* and malaria. This provided us the opportunity of studying the effect of HIV screening alone on the risk of other transfusion mediated infections without any other influencing variable during the study period unlike in most developed countries where, periodically, additional markers for HBV (in addition to HBsAg *eg* anti HBs, anti HBc) as well as markers for previously unrecognized agents like HCV, CMV have been included from time to time in the donor screening programs (Centers for Disease Control, 1991; Noal *et al*, 1994; Mirovic *et al*, 1971).

In the present study both groups of MT children, irrespective of their period of transfusion showed high prevalence of HBV and HCV infection (Table 1). Earlier studies on the nontransfused children carried out in our laboratory from the local community indicated prevalence of HBV to be as low as 8% (Chattopadhya *et al*, 1993) and that of HCV to be as low as 0.5% (unpublished observation), suggesting risk factors related to multiple transfusion were responsible for acquisition of HBV and HCV infections among the MT children in the present study.

Prevalence of HBV infection has been recorded to be high among the local blood donors in the city of Delhi in the present study as well as in an earlier study (Chattopadhya *et al*, 1997). Lack of any change in the prevalence of HBV markers despite signification reduction ($p < 0.001$) in the proportion of donors having sex with female CSWs during the donation period suggests acquisition of HBV infection among blood donors may occur through routes other than sexual (Zuckerman and Thomas, 1993). Nevertheless, irrespective of the mode of acquisition of HBV infection by blood donors, high prevalence of HBV infection in MT children in the present study may appear initially paradoxical in view of pre-existing guidelines for HBsAg screening in donated blood in this country. Limited studies in MT children from India employing additional markers of HBV infection (other than HBsAg), *viz* antiHBs and anti HBc have shown the actual load of HBV infection in MT children to be much higher (more than 10 times) than estimated on the basis of HBsAg detection alone reflecting the known limitations of HBsAg screening alone in detecting HBV virus infection (Gulati *et al*, 1992; Patwari *et al*, 1986; Udani *et al*, 1986; Williams *et al*, 1992).

Professional blood donors in the city of Delhi have been reported to have a high prevalence of HCV infection compared to voluntary donors, with a strong association with HIV infection (Chattopadhya *et al*, 1997). Mattson *et al* (1988) have demonstrated a reduction in the number of non A non B post transfusion hepatitis, now attributed mostly due to HCV, from 12 to 4 per thousand units of blood transfused between 1979 to 1986 in open heart surgery patients which was attributed to mandatory HIV testing, substantiating the known association of HCV with HIV (Mattson *et al*, 1988). Heterosexual promiscuity has not been established to be a common risk factor towards acquisition of HCV infection for which parenteral drug abuse, blood transfusion, tattooing and nosocomial spread are considered to be among the more common means (Van der Poel, 1994). The present study demonstrated that, apart from heterosexual promiscuity, high frequency of donation beyond permissible guideline, that recommends a minimum interval of 3 months between two consecutive donations (Saran and Ma-kroo, 1991), to be the most important risk behavior among the blood donors belonging to both groups. Thus it appears that HCV prevalence in these blood donors in our study, regardless of their periods of donation, could also be due to other recognized factors linked with repeated donations, *eg* nosocomial factors (Van der Poel, 1994). However, irrespective of the mode of acquisition, in the absence of any guidelines for screening HCV in donated blood, any load of HCV infection among blood donors is expected to be reflected among transfusion recipients, a factor that may account for higher prevalence of HCV infection among the MT children in our study as com-

pared to countries where HCV screening is mandatory in donated blood (Ebeling *et al*, 1991; Donahue *et al*, 1992).

The absolute risk of transmitting HDV in a transfusion setting carrying out HBsAg screening has been estimated to be less than one in 3,000 recipients, considering the frequency of association of HDV with HBV to be about 1 in 30 and HBV infection developing in less than 1 of 100 recipients of blood tested negative for HBsAg (Rosina *et al*, 1985; Aach and Kahn 1980). Several studies have shown little above 5% of HBsAg carriers in multitransfused thalassemics to be associated with HDV infection, a percentage similar to the degree of association of HDV infection found in HBsAg carriers in the general population thereby indicating environmental transmission to be a strong possibility for HDV infections (Smedile *et al*, 1981, 1983).

Nevertheless, our study demonstrates a decline in the load of infectious agents like CMV and HSV-2 in the MT children transfused after the introduction of mandatory screening of donated blood for HIV infection compared to those transfused prior to that. This correlates well with the recorded decline in the prevalence of these two infections among blood donors in the corresponding periods that could be attributed to safer sex practice following introduction of mandatory HIV screening in blood banks. None of the donors in either group gave a history of exposure to female CSWs prior to the period of donation, suggesting a possible link of visits to CSWs with the availability of money in exchange for donation in these remunerated donors (Chattopadhya *et al*, 1991). Admittedly, however, we could not utilize prevalence of antitreponemal antibody to substantiate this possibility due to relatively low positivity in both the groups of donors without any statistical difference, although such low prevalence correlated well with the small number of donors in both the groups visiting STD clinics. Low positivity of antitreponemal antibody observed in the present study is consistent with our earlier observations on HIV negative professional donors (Chattopadhya *et al*, 1997). However, such low prevalence of *T. pallidum* infection in HIV negative donors may not have any relation with the prevalence in the local population of female CSWs since in the same study mentioned above as many as 30% of HIV-1 positive donors showed evidence of *T. pallidum* infection (Chattopadhya *et al*, 1997). Such variation in the association of antitreponemal antibody and HIV positivity could be due to differences in factors like degree of heterosexual promiscuity in terms of number of exposures, risk category of sex partners, use of protective barrier precaution (*eg* condom) that we could not include in our study. However, evidence of *T. pallidum* infection could not be demonstrated in both the groups of sera from MT children analysed. This substantiates the known lability of the organism in storage conditions of blood and thereby indicating freedom from perceptible risk due to this agent in MT children even before the introduction of mandatory HIV screening in blood in this country (Weise, 1987).

Thus it appears that while change in the attitude of blood donors towards safer sex practice has been responsible for bringing down the risk of CMV and HSV-2 infections, such measure could not take care of the contribution of other possible risks factors, *eg* nosocomial factors towards acquisition of HBV, HCV and HDV infections by the donors while donating in unhygienic set-ups due to contamination from unsterile needles or contaminated surface. Such mode can independently contribute towards high prevalence of HBV, HCV and HDV infections in MT children, regardless of the passive mode of acquisition from infected donors through blood transfusion (Weise, 1987; Zuckerman and Thomas *et al*, 1993; Van der Poel, 1994). Alternatively, MT children could acquire these infections through nosocimial route while being transfused in set ups below the acceptable standard (Weise, 1987).

The authors feel that in the absence of any expansion in the existing guidelines to include HCV and markers for HBV infection in addition to those covered by the existing policy based on HBsAg screning alone, improvement in the standard of transfusion settings to prevent nosocomial infection is likely to be an important contributor towards the reduction of current load of HBV and HCV infections.

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