International Journal of Medical and Health Research
ISSN: 2454-9142, Impact Factor: RJIF 5.54
www.medicalsciencejournal.com
Volume 3; Issue 1; January 2017; Page No. 120-126

The study of hepatitis B and hepatitis C infection in patients and healthy blood donors

[1] Dr. Shamim Ahmad, [2] Dr. Ahsan Hamidi

[1] Tutor, Department of microbiology, Government medical college, Bettiah West Champaran, Bihar, India
[2] Assistant professor, Department of microbiology, Government medical college, Bettiah West Champaran, Bihar, India

Abstract
Introduction: Safe blood and blood products should be offered to all patients in need for blood transfusion. The objectives of the present study were to establish prevalence estimates for hepatitis B and hepatitis C virus infections as a foundation for safe blood transfusion in hospital, and to check the accuracy of the laboratory analysis used for hepatitis testing of blood donors in hospital.
Methods: A total of 1,200 blood samples collected from potential blood donors were tested by an enzyme immunoassay technique (EIA) for detection of hepatitis surface antigen (HBsAg), antibodies to hepatitis B core antigen (anti-HBc), and antibodies to hepatitis C antigen (anti-HCV). The EIA test outcome was validated by a chemiluminescent micro particle immunoassay technique (CMIA).
Results: The prevalence of HBsAg and anti-HBc in the study population was 11.4 per cent (95% CI 9.6 - 13.2) and 51.7 per cent (95% CI 48.8 - 54.5), respectively, the prevalences being higher in males than females. The prevalence of anti-HCV was 0.17 per cent. The test agreement between the EIA and CMIA techniques was high both for HBsAg detection ($\kappa = 0.91$; 95% CI: 0.83 - 0.99) and for anti-HBc detection ($\kappa = 0.89$; 95% CI 0.81 - 0.97). Compared to CMIA results, the positive and negative predictive values of the EIA tests were found to be 94.9 per cent (95% CI 87.5 - 98.6) and 97.5 per cent (95% CI 86.8 - 99.9) for HBsAg, and 92.4 per cent (95% CI 84.2 - 97.2) and 100 per cent (95% CI 91.2 - 100) for anti-HBc.
Interpretation & conclusions: The study shows that hepatitis B virus infection is endemic in local area and that almost population is or has been infected. Hepatitis C infection is rare, but false negative test results cannot be ruled out. Also, the results indicate that the EIA performance in blood donor screening may be sub-optimal, missing 2.5 per cent of hepatitis B virus carriers and falsely excluding more than 7 per cent of blood donors. As the prevalence of hepatitis B infection is high, occult hepatitis B infection may represent a threat to safe blood transfusion. Therefore, nucleic acid amplification testing for HBV should be considered for blood donor screening.

Keywords: Anti-HBc, blood donor screening, HBsAg - hepatitis B core antibody, hepatitis B surface antigen, hepatitis C virus, occult hepatitis B infection, safe blood transfusion

1. Introduction

Safe blood transfusions remain a challenge in resource-limited settings where blood-transmitted diseases are endemic. The transmission risk of hepatitis B virus (HBV) infection depends on the actual disease prevalence rate; where the prevalence is low the transmission risk is estimated at approximately 1:60,000; where HBV infection is endemic, the transmission rate is probably much higher [1-3]. Testing for hepatitis surface antigen (HBsAg) is in place in most low-income countries.

Also patients with HBV S gene mutants may escape detection by standard blood donor screening tests [4]. Besides, transmission can still occur during the initial window-period of an acute infection, or during late stages where virus is still present (HBV-DNA positive) though HBsAg is negative, so-called occult hepatitis B infection (OBI) [2, 5, 6].

OBI may originate from recovered infections with persistent low level viral replication, from escape mutants blocking export of antigen, or from a reduced HBV replication after co-infection with hepatitis C virus (HCV) infection [2, 7]. The presence of antibodies to hepatitis B core antigen (anti-HBc) indicates previous infection with HBV. In many Western countries the presence of anti-HBc excludes blood donation. However, due to limited resources and the potential exclusion of many blood donors, this routine is seldom practiced in low-income countries where HBV infection is endemic. There has been an increased focus on OBI lately, with several studies published both on the clinical significance of anti-HBc and OBI, and as a starting point for discussing the matter of safe blood transfusion.

The infectiousness of OBI cases is not clear although several studies report that exclusion of antiHBc positive blood probably decreases the rate of HBV transmission by blood transfusion [5, 8, 9]. The nucleic acid amplification (NAT) technology has greatly enhanced accuracy in identification of OBI cases, and NAT negative individuals are considered safe blood donors [10]. However, NAT testing is expensive, the NAT tests may not pick up mutated virus, cost-effectiveness may be low, and the technology may not be feasible in blood transfusion services where resources are scarce [4].

Prevalence studies of HBV and HCV have so far been conducted on relatively small study samples [11-15]. Consequently, the prevalence estimates are too imprecise to give evidence-based recommendations for blood donor screening. The aim of the present study was, therefore, to estimate accurate prevalence rates of HBV and HCV infections among potential blood donors in a rural area prior to setting up rural blood transfusion services.

2. Material & Methods

This work was done at the department of microbiology of

Government medical college, Bettiah West Champaran, Bihar. Regarding ethical aspect I had informed concerned authority of this college. For the purpose of establishing rural blood transfusion service, any healthy consenting adult between the age of 18 and 55 yr living permanently in the study area was considered a potential blood donor. Prior to the collection of blood samples, villagers were informed by local health authorities in media and community meetings that the study was linked with the introduction of safe blood transfusion service for the local population; that participation was voluntary and free of charge; that all participants would be informed of the test outcome and get medical advice and counseling accordingly. Individuals previously vaccinated for HBV were excluded from the study. After the informed consent forms were signed with the witness of blood collectors, participants were asked to fill in and sign registration forms before inclusion. A total of 1,200 persons who were willing to participate, and meeting selection criteria, were included in the study. Data on HBe antigen were not gathered. The sample size of 600 in each region enabled us to detect a difference of HBV prevalence of at least 10 per cent with significance level 5 per cent with twosided testing, test power at 90 per cent.

To assess local prevalence variations the blood samples were collected with stratified sampling procedure: 600 samples were collected from the villagers in hospital, and blood samples collected from the consenting individuals. With this sampling design, potential clustering of results at village level were considered and adjusted for the prevalence estimates.

To control the quality of sample processing, a subset of 321 serum samples was randomly selected for analysis by an automated chemiluminescent micro particle immunoassay (CMIA) technique (n=120 for each category, HBsAg, anti-HBc, and anti-HCV) (Fig. 1).

The subset sample size (n = 120) was estimated to detect test indicator differences of more than 5 per cent with 95% confidence interval, the subset being selected to get at a balance of 2/3 assumed test-positive units versus 1/3 assumed test-negative units. Except for a slight under-representation of females in the HBsAg subset, the demographical composition of the three subsets corresponded well with the total study population (Table I).

2.1 Methods

The EIA tests were performed according to the manufacturer's instructions. Blood samples (5 ml) were drawn in field from each study person by trained laboratory technicians and set aside for spontaneous coagulation for 30 min before centrifugation. After centrifugation, the serum was pipetted into two new tubes, cooled to 4°C in a portable cooler and analysed within three days by an EIA technique. The frozen serum samples from all participants were stored for one year in case further analysis should be required. The Monolisa test has a claimed sensitivity at 100 and specificity at 99.9 per cent for HBsAg; for anti-HBc the claimed sensitivity is 99.5 per cent and specificity 99.5 per cent; for anti-HCV the respective performance is claimed to be at 100 and 99.8 per cent[14].

The following confirmation of positive tests is recommended by the manufacturers: A neutralization test for reactive/ positive HBsAg samples; a recombinant immunoblot assay test or HCV-RNA polymerase chain reaction test for reactive/positive anti-HCV; alternatively anti-HBc assay for reactive/positive anti-HBc.

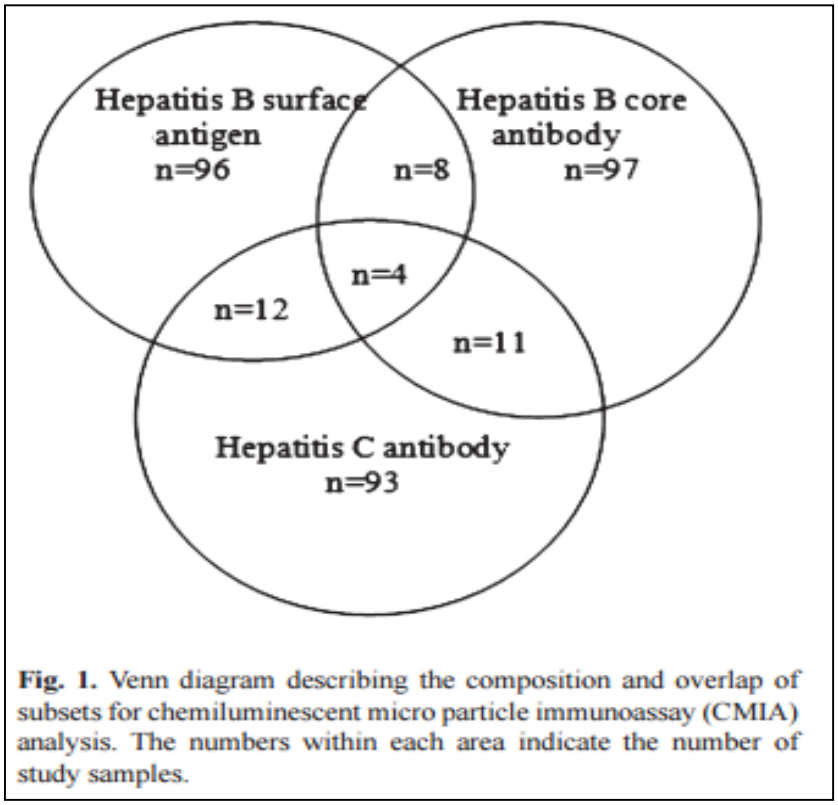

Fig. 1. Venn diagram describing the composition and overlap of subsets for chemiluminescent micro particle immunoassay (CMIA) analysis. The numbers within each area indicate the number of study samples.

Table I. Demographic factors in the study population and in chemiluminescent micro particle immunoassay (CMIA) subsets

	Study group (n=1,200)	Cam Thuy n=600	Trieu trach N=600	Subsets for CMIA analysis		
				HBsAg (n=120)	Anti-HBc (n=120)	Anti-HCV (n=120)
Gender ratio: female/male	730/470	401/199	329/271	55/65	65/55	64/56
Mean ± SD age (yr)	34.6 ± 8.6	34.2 ± 8.8	35 ± 8.4	34.4 ± 8.2	34.8 ± 8.6	35.0 ± 9.2

Samples with concentration values less than 0.05 IU/ ml were considered negative and those that had values ≥ to 0.05 IU/ml were considered positive. CMIA analysis of anti-HBc and anti-HCV assays is based on the ratio of signal to cut-off value (S/CO).

3. Results

The demographic characteristics of the study sample are presented in Table I and Fig. 2. The subsamples compared well to the main sample regarding age, and the subsamples were approximately equal for age and gender, while the main sample had a gender bias. The analyses showed that 11.4 per cent of study samples (137/1200, 95% CI 9.6 - 13.2) were positive for HBsAg, 51.7 per cent (620/1200, 95% CI 48.8 - 54.5) were anti-HBc-positive, while 9.5 per cent (114/1200, 95% CI 7.9 - 11.3) were positive for both HBsAg and anti-HBc. Further, 1.9 per cent (23/1200, 95% CI 1.2 -2.8) of the serum samples were positive for HBsAg and negative for anti-HBc; and 42.2 per cent (506/1200, 95% CI 39.4 - 45.0) negative for HBsAg and positive for anti-HBc.

There were only two positive anti-HCV samples (0.17%). A substantial age gradient with more positives with increasing age was found for anti-HBc, but not for HBsAg (Fig. 3). Gender was found to be important, as more men than women were positive for HBsAg (16.8%; 95% CI 13.4 - 20.2 versus 7.9%, 95% CI 6.0 - 9.9) and also for anti-HBc (57.0%; 95% CI 52.5-61.5 versus 48.2%; 95% CI 44.6 - 51.8).

Higher prevalence levels for both HBsAg and anti-HBc were found in remote rural area compared with the less remote area. However, these differences were mainly due to sampling bias (age, gender), as the area variable was without significant importance in the logistic models (Table II). As shown in Table III, that age and gender were substantial factors for anti-HBc variations while only gender was important for HBsAg. For both HBsAg and anti-HBc, higher prevalence was found for males versus females. Model fit was acceptable with a ROC area of 66 per cent for anti-HBc and 62 per cent for HBsAg.

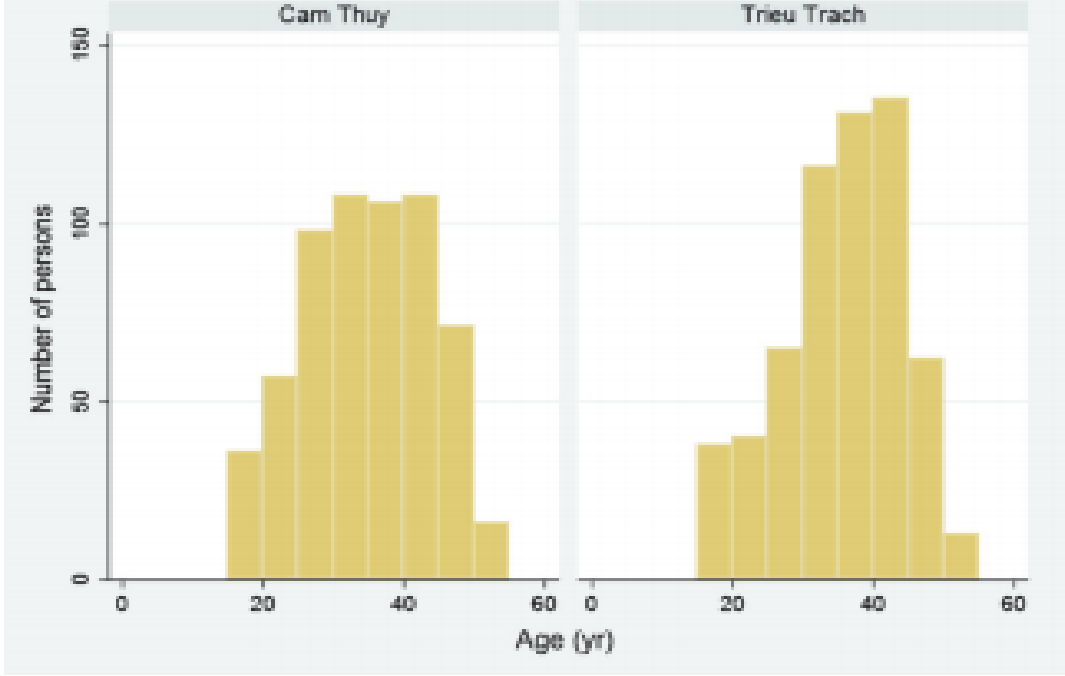

Fig. 2. The age distribution in the two study areas, Cam Thuy (more developed community, n = 600) and Trieu Trach (less developed community, n = 600).

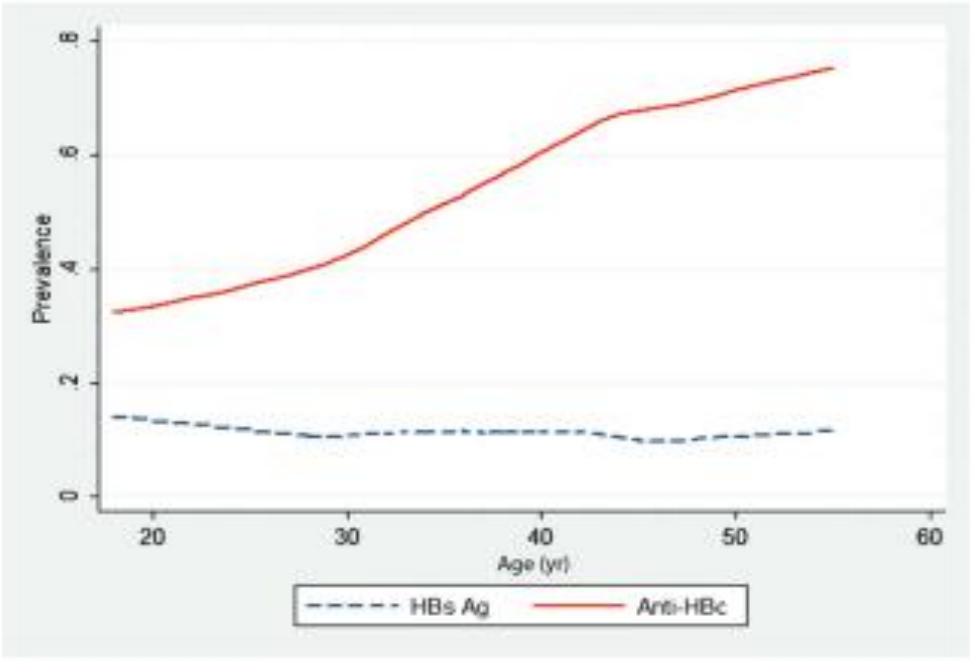

Fig. 3. The age profile for HBsAg and anti-HBc positive individuals established using the *lowess smoother* function in Stata.

The test agreement (Kappa) between the EIA and CMIA was 0.91 for HBsAg (95% CI: 0.83 - 0.99), and 0.89 for anti-HBc (95% CI: 0.81 - 0.97) (Table IV). Using CMIA test results as reference, the positive and negative predictive values were found to be 94.9 per cent (95% CI 87.5 - 98.6) and 97.5 per cent (95% CI 86.8 - 99.9) for HBsAg; and 92.4 per cent (95% CI 84.2 - 97.2) and 100 per cent (95% CI 91.2 - 100) for anti-HBc. Samples with difference in results for HBsAg and anti-HBc had close to cut-off values both in CMIA and EIA analysis. For the anti-HCV analysis the pattern was different, the two samples being clearly positive by CMIA and clearly negative by EIA (Table V).

4. Discussion

The present study documents high prevalence of HBV infection in the study area and thus confirms findings in previous smaller epidemiological studies conducted in rural as well as urban populations reporting prevalence rates of HBV infection in the range of 8 to 25 per cent [12-15]. Due to the size of the present study population, the prevalence estimates are strong with narrow confidence limits. Although there was a gender bias in the main sample, the study demonstrated that gender was a risk variable, men having a higher risk of HBV infection than women. The study also documents a high prevalence of anti-HBc (51.7%) in the study area.

The large number of samples being HBsAg negative and anti-HBc positive (42.2%) indicated that HBV infections have been endemic in the study area for some time. However, there may be large local prevalence variations as reported in studies of HBV infection [19]. Comparing two communities with different infrastructural levels, we found higher prevalence of anti-HBc in the less developed community. However, the difference was moderate (10%) and hardly of clinical significance. Of the 1,200 samples included in the present study, only two were classified as anti-HCV positive with EIA technique.

Table II. Adjusted prevalence estimates (%) of HBsAg, anti-HBc, and anti-HCV with 95% confidence intervals, established with the *svy* procedure in Stata, with village as primary sampling unit and area as stratifying variable, stratified over gender

	Adjusted prevalence estimates (%)		
	HBsAg	Anti - HBc	HCV
Total population (n=1,200)	11.4 (9.6 - 13.2)	51.7 (48.8 - 54.5)	0.17 (0.02 - 0.60)
Female	7.9 (6.0 - 9.9)	48.2 (44.6 - 51.8)	
Male	16.8 (13.4 - 20.2)	57.0 (52.6 - 61.5)	
Trieu Trach (n= 600)	12.0 (9.4 - 14.6)	56.7 (52.7 - 60.6)	0.17 (0.004 - 0.90)
Female	6.7 (4.0 - 9.4)	50.4 (45.0 - 55.9)	
Male	18.5 (13.8 - 23.1)	64.2 (58.5 - 70.0)	
Cam Thuy (n = 600)	10.8 (8.4 - 13.3)	46.7 (42.7 - 50.6)	0.17 (0.004 - 0.90)
Female	9.0 (6.2 - 11.8)	46.4 (41.5 - 51.3)	
Male	14.6 (9.6 - 19.5)	47.2 (40.3 - 54.2)	

Values in parentheses are 95% CI

Of these two, one was also positive for both HBsAg and anti-HBc; the other was HBsAg negative and anti-HBc positive. Two more anti-HCV positive samples were found with CMIA technique in Norway. The finding corresponds well with prevalence estimates at 0.4 per cent HCV infection reported.

Table III. A multivariable logistic model for HBsAg and anti-HBc expressing the influence of possible confounders on prevalence estimates, the results being expressed with 95% confidence intervals

Outcome	Variables	Odds Ratio	*P*
HBsAg	Age	0.99 (0.97 - 1.02)	0.695
	Male vs. female	2.34 (1.66 - 3.29)	< 0.001
	TT vs. CT	1.02 (0.56 - 1.84)	0.958
Anti-HBc	Age	1.06 (1.05 - 1.08)	< 0.001
	Male vs. female	1.41 (1.08 - 1.83)	0.011
	TT vs. CT	1.40 (0.87 - 2.27)	0.168

TT, Trieu Trach; CT-Cam Thuy

Table IV. Comparison of EIA and CMIA test outcomes

	EIA test outcomes			
	HBs Ag*		Ant-HBc*	
	Positive	Negative	Positive	Negative
CMIA positive	75	1	73	0
CMIA negative	4	39	6	40
Total	79	40	79	40
Sensitivity (%)	98.7 (92.9 - 100)		100 (95.1 - 100)	
Specificity (%)	90.7 (77.9 - 97.4)		87 (73.7 - 95.1)	
Positive predictive value (%)	94.9 (87.5 - 98.6)		92.4 (84.2 - 97.2)	
Negative predictive value (%)	97.5 (86.8 - 99.9)		100 (91.2 - 100)	
Agreement (kappa)	0.91 (0.83 - 0.99)		0.89 (0.81 - 0.97)	

*The result from one patient was missing from the CMIA analysis
The results expressed in observed numbers. Test sensitivity/specificity and positive/negative predictive values are given by 95% confidence intervals for EIA, using CMIA as reference method

Table V. Mismatched EIA and CMIA test results

HBsAg				Anti-HBc				Anti- HCV			
CMIA IU/ml	Result CMIA	Ratio EIA	Result EIA	CMIA S/CO	Result CMIA	Ratio EIA	Result EIA	CMIA S/CO	Result CMIA	Ratio EIA	Result EIA
0.04	NEG	1.38	POS	0.18	NEG	1.23	POS	2.12	POS	0.23	NEG
0.03	NEG	1.16	POS	0.74	NEG	1.53	POS	7.43	POS	0.59	NEG
0.03	NEG	1.10	POS	0.54	NEG	1.57	POS				
0.04	NEG	1.01	POS	0.62	NEG	1.19	POS				
0.58	POS	0.87	NEG	0.53	NEG	1.59	POS				
				0.60	NEG	1.27	POS				

S/CO: The ratio of signal to cut-off value in CMIA analysis

A recent study in rural Cambodia reports high prevalence rate of HCV antibodies (14.7%) [20]. Roughly 80 per cent of anti-HCV positive individuals are considered to be infectious[21]. The two clearly missed anti-HCV positive blood samples by EIA picked up by CMIA analysis, may be due to test properties. Both for HBV and HCV, there are genotype differences between Western countries and East Asia.

The test in use was developed by a Western company, and it may well be that this test did not identify the antibodies produced by the genotypes in the actual study population. Regardless of the reason, the study documented that the accuracy of hepatitis screening in potential blood donors was suboptimal. The false-negative HBsAg rate was 2.5 per cent, which increases the risk of transmitting HBV infections through blood products. Also the false-positive rate for anti-HBc of 7.6 per cent is unacceptably high and would exclude large numbers of potential blood donors.

An important problem is the risk of virus transmission from OBI donors. In the actual study population, the rate of HBsAg negative-anti-HBc positive participants was high (42%) which raised the question of how many of these were also OBI cases and potential transmitters of HBV infection? Accurate estimates cannot be given because the actual study participants due to resource restrictions could not be tested for HBV-DNA. The rate of HBV DNA detected in HBsAg-negative samples is reported to vary with the prevalence of HBV infection. Less than 5 per cent of HBsAg negative-anti-HBc positive blood donor samples are reported to have detectable HBV DNA in European countries with low HBV infection, whereas the corresponding rate is as high as 24 per cent in areas with high HBV prevalence [2, 8, 23].

A study conducted in China showed that the prevalence of occult HBV infection among HBsAg negative healthy individuals was 10.6 per cent24. On one hand, screening of blood donors for anti-HBc might reduce the risk of HBV transmission; but on the other hand, half the population would then be excluded from donation which has obvious consequences for blood product availability in the country.

Based on the present state of knowledge it is not possible to come up with actual risk estimates for HBV transmission by HBsAg negative-anti-HBc positive blood products in areas of high HBV endemicity. NAT studies of HBVDNA in HBsAg negative individuals are required to get a precise risk estimates for HBV transmission by HBsAg negative, anti-HBc positive blood products.

5. Conclusion

HBV is endemic in rural and constitutes a major public health problem. The study shows that half of the rural population has detectable anti-HBc antibodies and that the prevalence of potential transmitters of HBV infection is high. The prevalence of HCV seems to be low, however, genotype differences of the virus may affect the accuracy of test results. The study also indicated that the EIA performance in blood donor screening was sub-optimal. The screening tests should be validated by nucleic acid amplification test (NAT) technology.

6. References

1. Wang JT, Lee CZ, Chen PJ, Wang TH, Chen DS. Transfusion transmitted HBV infection in an endemic area: the necessity of more sensitive screening for HBV carriers. Transfusion 2002; 42:1592-9.
2. Hollinger FB. Hepatitis B virus infection and transfusion medicine: science and the occult. Transfusion 2008; 48:1001- 26.
3. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. N Engl J Med 1996; 334:1685-90.
4. Weber B. Recent developments in the diagnosis and monitoring of HBV infection and the role of the genetic variability of the S gene. Expert Rev Mol Diagn. 2005; 5:75-91.
5. Liu CJ, Lo SC, Kao JH, Tseng PT, Lai MY, Ni YH *et al*. Transmission of occult hepatitis B virus by transfusion to adult and pediatric recipients in Taiwan. J Hepatol 2006; 44:39-46.
6. Bhattacharya P, Chandra PK, Datta S, Banerjee A, Chakraborty S, Rajendran K *et al*. Significant increase in HBV, HCV, HIV and syphilis infections among blood donors in West Bengal, Eastern India 2004-2005: Exploratory screening reveals high frequency of occult HBV infection. World J Gastroenterol 2007; 13:3730-3.
7. Allain JP. Occult hepatitis B virus infection. Transfus Clin Biol. 2004; 11:18-25.
8. Behzad-Behbahani A, Mafi-Nejad A, Tabei SZ, Lankarani KB, Torab A, Moaddeb A. Anti-HBc & HBV-DNA detection in blood donors negative for hepatitis B virus surface antigen in reducing risk of transfusion associated HBV infection. Indian J Med Res. 2006; 123:37-42.
9. Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. Blood. 2002; 100:2637-41.
10. Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW. *et al*. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion. 2003; 43:788-98.
11. Nguyen VTT, McLaws ML, Dore GJ. Highly endemic hepatitis B infection in rural. J Gastroenterol Hepatol. 2007; 22:2093-100.
12. Nguyen VT, Law MG, Dore GJ. An enormous hepatitis B virus-related liver disease burden projected in Patna by 2025. Liver Int. 2008; 28:525-31.
13. Investigation into hepatits B virus infection among population in Nha Trang City. ln: Chien VC, Loan LTK, Tung LT, Nguyen HT, Van NT, Anh NT, *et al*, editors. Summary record of science works. Patna: Nha Tarang University; 1995-2001, 31-40.
14. Long HT, Van NT, Dat DT, Tran HQ, Cuong NV, Hao NH, *et al*. Situation of hepatitis B and C infections in Thanh Hoa. J Prev Med 1999; 2:5-10.
15. Duc DD. The epidemiology of hepatitis virus in Patna. J Pract Med 1997; 339:1-3.
16. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. editors. Manual of clinical microbiology. Washington DC: ASM Press; 2007, 1424-36.
17. Abbott diagnostics. Products/instruments. Available from: http://www.abbottdiagnostics.com/Products/Instruments_by_Platform/systests.cfm?system=architect&suffix=i2000, accessed on January 11, 2009.
18. Altman DC. Practical statistics for medical research. London: Chapman & Hall/CRC, 1999.
19. Ishida T, Takao S, Wannapa S, Tiwawech D. Prevalence of hepatitis B and C virus infection in rural ethnic populations of Northern Thailand. J Clin Virol. 2002; 24:31-5.
20. Ol HS, Bjoerkvoll B, Sothy S, Heng YV, Hoel H, Husebekk A. *et al*. Prevalence of hepatitis B and hepatitis C virus infections in potential blood donors in rural Cambodia. SE Asian J Trop Med Public Health 2009; 40:963-71.
21. Lemon SM, Walker C, Alter MJ, Yi M. Hepatitis C virus. In: Knipe DM, Howley PM, editors. Fields virology. Philadelphia: Lippincott Williams & Wilkins; 2007, 1253-305.
22. Valsamakis A. Molecular testing in the diagnosis and management of chronic hepatitis B. Clin Microbiol Rev 2007; 20:426-39.
23. Garcia-Montalvo BM, Farfan-Ale JA, Acosta-Viana KY, Puerto-Manzano FI. Hepatitis B virus DNA in blood donor with anti-HBc as a possible indicator of active hepatitis B virus infection in Yucatan, Mexico. Transfus Med. 2005; 15:371-8.
24. Fang Y, Shang QL, Liu JY, Li D, Xu WZ, Teng X *et al*. Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. J Infect 2009; 58:383-8.