International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

The importance of screening for anti HBc antibodies and hepatitis B virus DNA in healthy HBsAg negative blood donors in Morocco

Ghizlane Khalid [1], Marouane Aouji [1], Hicham El Annaz [2], Jaouad Rochdi [3], Abdelkader Belmekki [3], Rachid Bengueddour [1]*

[1] *Faculty of Sciences, University of Ibn Tofail, Kenitra, Morocco.*

[2] *Faculty of Medicine and Pharmacy, Mohamed V University, Rabat, Morocco.*

[3] *Faculty of Medicine and Pharmacy, Mohamed V University, Rabat, Morocco.*

Abstract

The hepatitis B virus represents a major public health issue, despite effective serological screening techniques. The main objective of our study is to determine the prevalence of post-transfusion HBV in HBsAg negative blood donors during a window period or following occult HBV infections by testing for anti-HBC antibody and HBV DNA screening to improve blood transfusion safety. A cross-sectional study carried out on 1000 young blood donors all are single males from different regions of Morocco, over a period from March 2018 to June 2018 at the Blood Transfusion Center located within Mohammed V Military Hospital of Instruction in Rabat (Morocco). All study participants were over 18 years old and tested HBsAg negative. The pre-donation medical interview was done to select the subjects at risk. The microparticulate chemiluminescence immunoassay (CMIA) was used for serological analysis. All the donors were male and the prevalence of anti HBc was 1.5% (n = 15). The average age of patients with anti-HBc was 26.00 ± 1.254 years (minimum = 24 and maximum = 28). Slightly more than 5.22% of regular donors (n = 134) were anti HBc positive. These factors have been shown to be significantly associated with the presence of anti HBc. The PCR test did not detect any HBV DNA. Despite the progress made in transfusion medicine, blood transfusion centers are called upon to reduce the transmission of post-transfusion hepatitis B by screening for anti-HBc antibodies or Viral DNA in blood donors.

Keywords: Hepatitis B, anti-HBc, HBsAg, blood donors, HBV DNA, prevalence of HBV.

Full length article **Corresponding Author*, e-mail: ghizlanekhalid9@gmail.com

1. Introduction

Despite the availability of a vaccine and antiviral treatments, hepatitis B infection remains a serious global public health problem that affects more than two billion people worldwide [1], 248 million of these people are chronically infected (defined as positive hepatitis B surface antigen [HBsAg] beyond 6 month) [2]. In Morocco, the epidemiology of viral hepatitis is not precisely well known. No national serological study has been carried out to estimate the real extent of the problem linked to these infections in the general population. According to WHO data it is considered as an area of intermediate endemicity (the prevalence of HVB at 2% in the general population) [3, 4]. Hepatitis B virus (HBV) is an enveloped DNA virus belonging to the Hepadnaviridae family, infection with this virus could lead to acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma, posing a huge burden for public health [5]. The HBV virus transmission includes a variety of routes including mother-to-child transmission (vertical transmission), sexual transmission, exposure to infected blood through the use of contaminated needles and syringes, and blood transfusion (infected blood or its components) [6]. The latter route is very important because blood transfusion is a frequent therapeutic procedure, with around 108 million units of blood collected each year worldwide [6]. Although the incidence of transfusion-transmitted hepatitis B has been steadily reduced over the past four decades [7], through the recruitment of voluntary donors, selection of donors based on a behavioral risk assessment [8] can still occur from apparently healthy blood donors. This may be attributed to the screening tests inability to detect HBsAg during the window period or following occult HBV infections [9]. The search for anti-HBc Abs is being carried out in several countries where the prevalence of hepatitis B is low: it is used as a contact marker with HBV in cases where HBsAg is not detected leading to a decreased risk of post-transfusion HBV infection [10]. Serologic reactivity to anti-HBc Ab may indicate active

exposure to HBV, chronic infection or resolutive infection [10].

Meanwhile HBV DNA could play a central role in revealing occult HBV infections or chronic carriers thus shortening the window period [11]. In Morocco, the HBsAg test is the only screening method in blood transfusion centers for the prevention of HBV transmission during a blood transfusion is based, our study is to determine the prevalence of post-transfusion HBV in HBsAg negative blood donors during a window period or following occult HBV infections by testing for anti-HBC antibody and HBV DNA screening to improve blood transfusion safety.

2. Materials and methods

This is a retrospective study carried out at the Blood Transfusion Center (CTS), located within the Mohammed V Military Hospital of Instruction in Rabat (Morocco). This center supervises all components of the transfusion chain from collection, screening for transfusion-transmissible diseases (ITT) and distribution of labile blood products.

2.1. Blood donors

The study population comprises 1000 military donors all are single males 21 years and a maximum of 31 years from different regions of Morocco, over a period from March 2018 to June 2018. The pre-donation medical interview was done to select the subjects at risk.

2.2. Methods

The collected blood was centrifuged and the serum was separated and subdivided into two aliquots stored at -80 °C for further processing. These samples are used for serological screening for anti-HBc markers using the microparticulate chemiluminescence immunoassay (CMIA) in the Architect platform (Abbott Laboratories, Wiesbaden, Germany). All the selected negative donations for HBsAg were tested by PCR to detect HBV DNA using the AmpliPrep / COBAS ® TaqMan ® HBV kit, v2.0 (Roche Diagnostics).

2.3. Statistical analyze

After filtering the data collected on Excel, we transferred them to SPSS software (trial version). Qualitative variables are expressed in frequencies and measurable variables as average. Tests such as Khi-square and Fisher tests were applied. Biological indicators such as prevalence, sensitivity, specificity, odds ratio, were all calculated.

3. Results

3.1. Sociodemographic characteristics of blood donors

The 1000 young blood donors, all are single males, representing all the regions of Morocco with more than 50% from the regions of FES-Meknes (n = 260) and Rabat-Sale-Kenitra (n = 250). They almost reached the secondary education level. The average age was 24.99 ± 0.05 years, with a minimum of 21 years and a maximum of 31 years. The distribution of these young recruits according to the answers to the question "is it your first-time donating blood" shows that 86.3% (n = 863) are first time donors compared to 13.7% (n = 137) (Table 1).

3.2. Serological and Molecular Testing

The Microparticulate Chemiluminescence Immunoassay (MIC) revealed 15 anti-HBc seropositive donors, representing a prevalence of 1.5% of which 8 are new donors and 7 are not. Finally, all the selected donations negative for HBsAg were tested negative by PCR test (Table 2). The khi2 test of independence between the anti HBc test and the region of origin shows a significant association (Khi2 = 18.13; $p < 0.05$). Indeed, out of the 15 anti-HBc positive donors, 7 were from Fès-Meknes, 3 were from Rabat-Sale-Kenitra, and 5 donors from other regions of Morocco. The results from the analysis of variance with a single classification criterion "anti-HBc test effect" are presented in table (2). Fisher's test shows a significant difference between the mean age of the two categories (Fisher = 6.58; $p < 0.01$). Indeed, the average age of HBc-negative donors is 24.99 ± 1.517 years (minimum = 21 and maximum = 31) while the average age of HBc-positive donors is 26.00 ± 1.254 years (minimum = 24 and maximum = 28). The table above presents the results of clinical indicators between the categories of the anti-HBc test and whether or not they are new donors. The Khi2 test shows that these two factors are strongly associated (Khi2 = 14.26; $p < 0.000$), with a Yule Q coefficient of 0.71. In fact, 5.22% (sensitivity) of individuals with anti HBc among non-new donors (n = 134). While 99.06% (specificity) were found to be negative by the anti HBc test among new donors (n = 855). Calculation of the Youden Index = (sensitivity + specificity - 1) shows that this test is effective with an index of 0.04. This value remains far from 1 but it is accepted for a squad that is not high enough. The attributable risk between seropositive and seronegative non-new donors is 33.63% (Odds ratio = 5.84. Confidence interval at 5% error is 2.08-16.38). The distribution of donors according to antecedent (vaccine against HBV) shows that 5.6% have already been vaccinated against this virus compared to 94.4% who have not. The breakdown between the presence of anti HBc and the previous vaccine shows that no anti HBc positive donors have been previously vaccinated against HBV. On the other hand, the cross-study between the new donors and the antecedent shows that the latter two are significantly associated (Khi2 = 3.49; $p < 0.037$). In addition, 5.36% (n = 3) of donors who have already been vaccinated against HVB (n = 56) have already been shown to participate in a blood donation (Table 2).

4. Discussions

Since its introduction, serological screening has remained a very important practice in the detection of viruses in the blood of donors (transfusion). However, the absence of HBsAg does not exclude the risk of occurrence of post-transfusion hepatitis B [12]. In fact, a combination of HBSAg and Anti-HBC screening is necessary for a final decision that a donation is exclusively negative [13,14,15]. The inclusion of anti-HBc testing for donor screening will permanently eliminate potentially HBV-infected donations. Thus, although many donations are rejected, this rejection will be valuable in reducing the risk of HBV transmission and its potential consequences, especially in immunocompromised recipients [16]. But performing both tests does not completely eliminate the risk of HBV transmission to blood recipients. Given the above considerations, genomic screening (NAT) can identify early infection, long before HBsAg and anti-HBc reach the blood circulation [6].

Table 1: Sociodemographic characteristics

Variants	Modalities	Male n=1000	Female n=0
Age (year)		24.99 ± 0.048	-
Region	Fes / Meknes	25% (n=260)	-
	Rabat / Sale / Kenitra	25% (n=250)	-
	Other regions of Morocco	50% (n=490)	-
Frequent donor (at least once)	Yes	13.7% (n=137)	-
	No	86.3% (n=863)	-
HVB vaccine	Yes	5.6% (n=56)	-
	No	94.4% (n=944)	-

Table 2: Serological and molecular testing for HBV

	Modalities	Presence of Anti HBc (n=15; 1, 5%)	Presence of Anti HBC-985 (98,5%)	P
Age (years)		26.00 ± 1.254	24.99 ± 1.517	< 0.01
Regions	Fes / Meknes	7	253	< 0.05
	Rabat / Sale / Kenitra	3	247	
	Other regions of Morocco	5	495	
Donor	Yes	8	847	< 0.0001
	No	7	127	
Anti HVB vaccine	Yes	0	56	<0.037

In this regard, it is proposed that the implementation of genomic screening (NAT) in high and low prevalence countries could lead to substantial safety given the window period. In view of the above considerations, it is proposed that the implementation of genomic screening (NAT) in high and low prevalence countries could lead to substantial safety given the window period and the risk of occult infections by transfusion-transmitted hepatitis B [17]. Our work consists in giving an anti-HBc positive donor prevalence, which was 1.5%, much: in Egypt (11%) [18] and 22% in Lebanon [19]. This prevalence was 28.18% in a study conducted in New Jersey on Korean American adults [20]. The rate reaches 4.9% in Iran [9]. Prevalence raised, 1.25% in Saudi Arabia [21], 1.59% in Germany [22], 4.86% in Italy [23], 7.5% in India [24]. This rate is the highest in Sudan [25] (90.5%). Our study showed that the factors of region of origin, age, new donor and history of the vaccine were determining factors in the negativity or the positivity of tests. These results are comparable to those found by studies conducted in Egypt [18] and other countries around the world [9]. In this study, no HBV DNA in the 1000 negative HBsAg blood samples was detected, regardless of positive or negative anti-HBc test results. This may be due to the size of the sample, the power of the study, or the composition of the target population. This low score is surely related to the nature of young donors participating in our study, the improvement of donor selection procedures as well as by the integration, in the national program, of the infants HBV vaccination in 1992. In addition, vaccination program in 1999, hepatitis B immunoglobulin treatment and HBV vaccine for neonates of mothers infected with HBV at delivery could also contribute to the significant reduction of vertical transmission or perinatal HBV [4].

5. Conclusions

Despite the progress made in transfusion medicine, blood transfusion centers in Morocco need called to increase their efforts to reduce the transmission of post-transfusion hepatitis B by introducing anti-HBc antibody screening or viral DNA which is highly sensitive and more recommended for blood donors. This is challenging the health system in low-income countries due to its cost.

State of knowledge on the subject

Despite considerable improvements in blood donation and the development of more advanced screening methods, transfusion-transmitted infectious agents such as the hepatitis B virus still pose a threat to blood safety.

Contribution of our study to knowledge about the subject

No national serological survey has been conducted to estimate the true extent of hepatitis B transmission in the general population. Screening for HBSAg with anti HBC and HBV DNA is necessary to eliminate the risk of transmission of occult hepatitis B during the window period to recipients. This study shows that the prevalence of anti HBC Ab in military blood donors in Morocco is lower than that observed in Egypt, Iran, Sudan and Italy, but higher than that observed in Saudi Arabia.

Competing interests

The authors declare no competing interests.

Authors' contributions

All authors contributed to the development of this work, read and approved the final version of the manuscript.

References

[1] D. Candotti, S. Laperche. (2018). Hepatitis B virus blood screening: need for reappraisal of blood safety measures? Frontiers in Medicine. 5:29.

[2] N.P. Nelson, P.J. Easterbrook, B.J. McMahon. (2016). Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. Clinics in Liver Disease. 20(4):607-628.

[3] F. André. (2000). Hepatitis B epidemiology in Asia, the middle East and Africa. Vaccine. 18: S20–S22.

[4] National viral hepatitis seroprevalence. http://www.sante.gov.ma. 18 juin 2022,13 :26.

[5] D. Fopa, D. Candotti, C.T. Tagny, C. Doux, D. Mbanya, E.L. Murphy, H.I. Kenawy, F. El Chenawi, S. Laperche. (2019). Occult hepatitis B infection among blood donors from Yaoundé, Cameroon. Blood Transfusion. 17(6): 403.

[6] F.M. Alzahrani, S.S. Shaikh, A.I. Alomar, S. Acharya, N. Elhadi. (2019). Prevalence of hepatitis B virus (HBV) among blood donors in Eastern Saudi Arabia: results from a five-year retrospective study of HBV seromarkers. Annals of laboratory medicine. 39(1):81.

[7] H. Kumar, P.K. Gupta, M. Jaiprakash. (2007). The role of anti-HBc IgM in screening of blood donors. Medical Journal Armed Forces India. 63(4):350–352.

[8] Z.N. Said, M.H. El Sayed, I.I. Salama, E.K. Aboel-Magd, M.H. Mahmoud, M. El Setouhy, F. Mouftah, M.B. Azzab, H. Goubran, A. Bassili. (2013). Occult hepatitis B virus infection among Egyptian blood donors. World journal of hepatology. 5(2): 64.

[9] G. Karimi, M. Zadsar, N. Vafaei, Z. Sharifi, M. Falah Tafti. (2016). Prevalence of antibody to Hepatitis B core antigen and Hepatitis B virus DNA in HBsAg negative healthy blood donors. Virology journal. 13(1):1-6.

[10] B.R. Tiwari, P. Ghimire, S.R. Kandel, M. Rajkarnikar. (2010). Seroprevalence of HBV and HCV in blood donors: A study from regional blood transfusion services of Nepal. Asian journal of transfusion science. 4(2):91.

[11] A. Esposito, C. Sabia, C. Iannone, G.F. Nicoletti, L. Sommese, C. Napoli. (2017). Occult Hepatitis Infection in Transfusion Medicine: Screening Policy and Assessment of Current Use of Anti-HBc Testing. Transfus Med Hemother. 44(4):263–272.

[12] L.N. Shih, J.C. Sheu, J.T. Wang, G.T. Huang, P.M. Yang, H.S. Lee, J.L. Sung, T.H. Wang, D.S. Chen. (1990). Serum hepatitis B virus DNA in healthy HBsAg-negative Chinese adults evaluated by polymerase chain reaction. Journal of medical virology. 32(4): 257-260.

[13] G.B. Schreiber. (1996). The risk of transfusion-transmitted viral infections. The Epidemiology Donor Study. New England Journal of Medicine. 334(26):1734–1735.

[14] R.A. Sacher, G.B. Schreiber, S.H. Kleinman. (2000). Prevention of transfusion-transmitted hepatitis. The Lancet. 355(9201):331–332.

[15] C. Matsumoto, K. Tadokoro, K. Fujimura, S. Hirakawa, S. Mitsunaga, T. Juji. (2001). Analysis of HBV infection after blood transfusion in Japan through investigation of a comprehensive donor specimen repository. Transfusion. 41(7):878–884.

[16] R. Panigrahi, A. Biswas, S. Datta, A. Banerjee, P.K. Chandra, P. K. Mahapatra, B. Patnaik, S. Chakrabarti, R. Chakravarty. (2010). Anti-hepatitis B core antigen testing with detection and characterization of occult hepatitis B virus by an in-house nucleic acid testing among blood donors in Behrampur, Ganjam, Orissa in southeastern India: implications for transfusion. Virology Journal. 7(1):1–7.

[17] J.-P. Allain, L. Cox. (2011). Challenges in hepatitis B detection among blood donors. Current opinion in hematology. 18(6): 461-466.

[18] A.R. El-Zayadi, E. Ibrahim, H. Badran, A. Saeid, N. Moneib, M. Shemis, R. Abdel-Sattar, A. Ahmady, A. El-Nakeeb. (2008). Anti-HBc screening in Egyptian blood donors reduces the risk of hepatitis B virus transmission. Transfusion medicine. 18(1): 55-61.

[19] S. Ramia, F. Ramlawi, M. Kanaan, S. Klayme, R. Naman. (2005). Frequency and significance of antibodies against hepatitis B core (anti-HBc) antigen as the only serological marker for hepatitis B infection in Lebanese blood donors. Epidemiology & Infection. 133(4):695–699.

[20] C.S. Hyun, S. Lee, W.R. Ventura. (2019). The prevalence and significance of isolated hepatitis B core antibody (anti-HBc) in endemic population. BMC research notes. 12(1):1–5.

[21] A.-R.N. Zekri, A.A. Awlia, H.E. Mahalawi, E.F. Ismail, G.M. Mabrouk. (2002). Evaluation of blood units with isolated anti HBC for the presence of HBV DNA. Disease markers. 18(3): 107-110.

[22] M. Schmidt, C.M. Nübling, H. Scheiblauer, M. Chudy, L.A. Walch, E. Seifried, W.K. Roth, M.K. Hourfar. (2006). Anti-HBc screening of blood donors: a comparison of nine anti-HBc tests. Vox sanguinis. 91(3):237–243.

[23] P. Manzini, M. Girotto, R. Borsotti, O. Giachino, R. Guaschino, M. Lanteri, D. Testa, P. Ghiazza, M. Vacchini, F. Danielle. (2007). Italian blood donors with anti-HBc and occult hepatitis B virus infection. Haematologica. 92(12): 1664-1670.

[24] M. Asim, R. Ali, L.A. Khan, S.A. Husain, R. Singla, P. Kar. (2010). Significance of anti-HBc screening of blood donors and its association with occult hepatitis B virus infection: Implications for blood transfusion. Indian Journal of Medical Research. 132(3):312–317.

[25] Abd El Kader Mahmoud, O., Abd El Rahim Ghazal, A., El Sayed Metwally, D., Elnour, A.M., & Yousif G. E. A. (2013). Detection of occult hepatitis B virus infection among blood donors in Sudan. Journal of The Egyptian Public Health Association. 88(1):14-18.