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Original Article

Impact of Multiplex PCR in Reducing the Risk of Residual Transfusion-Transmitted Human Immunodeficiency and Hepatitis B and C Viruses in Burkina Faso

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Abstract. ***Background and Objective:*** **The improved performance of serological tests has significantly reduced the risk of human immunodeficiency and hepatitis B and C viruses transmission by blood transfusion, but there is a persistence of residual risk. The objective of this study was to evaluate the impact of multiplex PCR in reducing the risk of residual transmission of these viruses in seronegative blood donors in Burkina Faso.**
Methods: **This cross-sectional study was conducted from March to September 2017. The serological tests were performed on sera using ARCHITECT$_{SR}$ *i*1000 (Abbot diagnosis, USA). Detection of viral nucleic acids was performed by multiplex PCR on mini-pools of seronegative plasma for HBV, HCV and HIV using SaCycler-96 Real Time PCR v.7.3 (Sacace Biotechnologies). Multiplex PCR-positive samples from these mini-pools were then individually tested by the same method.**
Results: **A total of 989 donors aged 17 to 65 were included in the present study. "Repeat donors" accounted for 44.79% (443/989). Seroprevalences for HIV, HBV, and HCV were 2.53% (25/989), 7.28% (72/989) and 2.73% (27/989), respectively. Of the 14 co-infections detected, HBV/HCV was the most common with 0.71% (7/989) of cases. Of 808 donations tested by multiplex PCR, 4.70% (38/808) were positive for HBV while no donation was positive for HIV or HCV.**
Conclusion: **Our study showed a high residual risk of HBV transmission through blood transfusion. Due to the high prevalence of blood-borne infections in Burkina Faso, we recommend the addition of multiplex PCR to serologic tests for optimal blood donation screening.**

Keywords: HIV, HBV, HCV, multiplex PCR, Transfusion, Burkina Faso.

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Introduction. Optimal transfusion-transmitted infections safety remains a permanent concern in the world and especially in sub-Saharan Africa. In transfusion practice, priority is given to the screening of three major pathogenic viruses that are Human Immunodeficiency Virus (HIV) and Hepatitis B (HBV) and C (HCV) Viruses. The highest prevalence of these viruses is found in sub-Saharan Africa,[1,2] where 12.5% of transfused patients are at risk for post-transfusion hepatitis and 5-10% at risk of HIV infection.[1,3] In Burkina Faso, previous studies have reported seroprevalences between 0.5%-3%, 8%-15% and 1%-9% respectively for HIV, HBV and HCV in the general population and in blood donors.[4-9]

Today, the risk of transmission of these viruses through transfusion has markedly decreased due to the application of preventive measures and improved performance of serological tests. Nevertheless, there is still a persistence of a residual risk mainly related to the serological window periods characterized by low-level serological viral markers, which are usually undetectable by conventional serological assays.[10] In order to ensure blood safety, the World Health Organization (WHO) recommends the recruitment of voluntary, regular and unpaid donors selected from low-risk populations.[11]

In Burkina Faso, since its operationalization in 2005, Regional Blood Transfusion Center of Ouagadougou (CRTS/O) has continuously improved its blood transfusion safety policy by applying measures aimed at reducing the residual risk of transfusion, namely: medical selected donors, recruitment of voluntary, regular and unpaid donors and the use of fourth-generation serological tests for the biological qualification of blood donations.

With a view to optimal transfusion safety research, Burkina Faso is now considering the introduction of PCR in the screening of blood donations. The application of this technology in blood transfusion in developed countries has made it possible to reduce the residual transfusion risk associated with the serological window.[12-14] Burkina Faso, a developing country (DC) has a high endemicity of these transfusion-transmitted viral infections but has very little data on the impact of multiplex PCR in our context. The present study was conducted at the CRTS/O to evaluate the impact of multiplex PCR in reducing the risk of residual transfusion-transmitted HIV, HBV and HCV infections in blood donors seronegative for these three viruses in Burkina Faso.

Material and Methods.

Type and population of study. This was a cross-sectional study that took place from March to September 2017 at the CRTS/O. The study population consisted of blood donors of both genders accepted for fixed-site blood donation and mobile collection at the CRTS/O at the end of the medical selection. Medical screening was performed by qualified health professionals based on a standardized pre-donation interview questionnaire designed to gain insight on risk behaviors for HIV, HBV and HCV infections. Each donor freely agreed to participate in the study. A "repeat donor" was any donor who had already given at least one blood donation before this study. Otherwise, he was considered "first-time donor".

Ethical considerations. Ethical approval for the study was obtained from Ethics Committee for Health Research of Burkina Faso (deliberation n° 2015-6-080). Written informed consent was provided by all study participants.

Sampling. Venous blood specimens were collected from blood donors on a red-top and EDTA tubes. After centrifugation at 1500 g for 20 minutes, aliquots of serum and plasma were performed within 6 hours after samples collection for laboratory analyzes.

Screening for HIV, HBV and HCV serological markers. The serological markers of HIV, HBV and HCV were investigated on donor sera by chemiluminescent microparticle immunoassay (CMIA) using ARCHITECT *i*1000 SR (Abbott Diagnosis, USA). The p24 antigen and antibodies against HIV1/2 were simultaneously investigated using the ARCHITECT HIV Ag/Ab Combo Kit (Abbott, Wiesbaden Germany). HBV surface antigen (HBsAg) and anti-HCV antibodies were respectively determined using the ARCHITECT HBsAg Qualitative II (Abbott, Ireland, Sligo Ireland) and ARCHITECT Anti-HCV (Abbott GmbH & Co.KG, Wiesbaden Germany).

Detection for viral genomes of HIV, HBV and HCV. The detection of viral nucleic acid was

performed sequentially on the samples obtained exclusively from blood donors who tested seronegative for the three viruses (HIV, HBV, HCV). A first multiplex PCR was carried out on mini-pools of 8 plasma samples each. The mini-pools detected positive were reconstituted into 2 mini-pools of 4 plasma samples for a second multiplex PCR. Finally, an individual PCR assay was performed on the samples from positive mini-pools to the second PCR.

Constitution of mini-pools. Seronegative tested samples were divided into mini-pools of 8 plasma samples each. Each mini-pool consisted of 160 µL of plasma from each donation (i.e. 1.28 mL of plasma per mini-pool). Mini-pools of 8 plasma tested positive at the first assay were reconstituted into mini-pools of 4 plasma samples of 100 µL of each donation (i.e. 400 µL per mini-pool).

Extraction and amplification by multiplex PCR in real time. Molecular analysis was performed at the laboratory of Molecular Biology and Genetics (CERBA/ LABIOGENE). Extractions of viral nucleic acid were performed with 100 µL of mini-pool plasma using the Ribo-sorb sacace™ Kit (Sacace Biotechnologies®, Como Italy). PCR amplification was performed on the SaCycler-96 Real Time PCR v.7.3 (Sacace Biotechnologies) with the HCV/HBV/HIV Real-TM sacace™ multiplex kit (Sacace Biotechnologies®, Como, Italy). The PCR was carried out using a reaction volume of 25 µL (10 µL of DNA/RNA and 15 µL of the mix). The SaCycler-96 real-time multiplex PCR amplification was performed according to the following program: 1 cycle of 95°C for 15 s follows by 46 cycles of 95°C for 15 s and 60°C for 40 s. The sensitivity of the HCV/HBV/HIV Real-TM kit was respectively 10 IU/mL, 5 IU/mL and 20 copies/mL for HCV, HBV and HIV. After the first two amplifications, individual real-time PCR for HBV-positive samples of the mini-pools was performed to find the positive sample (s) from each pool. For this step the sacace™ Ribo-Sorb Silica kit was used for extraction and the sacace™ HBV Real-TM Qual kit was used for amplification according to the manufacturer's recommendations.

Statistical analyzes. The data were analyzed using the Epi Info version 7 software. The chi-square test was used for comparisons and any value was considered statistically significant for $p \leq 0.05$.

Results.

Seroprevalences of HIV, HBV and HCV according to the socio-demographic characteristics of blood donors at the CRTS/O. A total of 989 blood donors were included in this study. The age of donors ranged from 17 to 65 years with an average of 27.29 +/- 8.81 years. The 21 to 30 age group was the most represented with 55.31% (547/989) of donors with a sex ratio (M/F) of 1.96 in the study population. Of all blood donors, 55.21% (546/989) were first-time donors and 66.90% (632/989) were recruited from mobile collection sites (**Table 1**).

Table 1. Seroprevalences of HIV, HBV and HCV according to socio-demographic characteristics of blood donors at CRTS / O.

Parameters	Donations N = 989	HIV (n = 25)	*P value*	HBV (n = 72)	*P value*	HCV (n = 27)	*P value*
Age groups							
< 20	199 (20.12)	4 (2.01)		11 (5.53)	*0,191*	3 (1.51)	*0,606*
21-30	547 (55.31)	13 (2.38)	*0.567*	47 (8.59)		18 (3.29)	
31-40	134 (13.55)	6 (4.48)		11 (8.21)		3 (2.24)	
41-50	86 (8.70)	2 (2.33)		3 (3.49)		3 (3.49)	
> 50	23 (2.33)	0 (0.0)		0 (0.0)		0 (0.0)	
Gender							
Male	655 (66.23)	15 (2.29)	*0.504*	53 (8.09)	*0.168*	24 (3.66)	***0.011***
Female	334 (33.77)	10 (2.99)		19 (5.69)		3 (0.9)	
Donor type							
First-time donors	546 (55.21)	14 (3.16)	*0.290*	41 (9.26)	***0.029***	16 (3.61)	*0.130*
Repeat donors	443 (44.79)	11 (2.01)		31 (5.68)		11 (2.01)	
Collection site							
Fixed site	357 (36.10)	5 (20)	< ***0.001***	19 (26.39)	< ***0.001***	5 (18.52)	< ***0.001***
Mobile site	632 (63.90)	20 (80)		53 (73.61)		22 (81.48)	

Table 2. Distribution of HBV DNA according to the socio-demographic characteristics of the 38 donors screened positive for multiplex PCR.

Characteristics	Positive HBV PCR (n = 38)	p-value
Gender		
Male	25 (65.8)	0.051
Female	13 (34.2)	
Age group		
< 20	13 (34.2)	0.124
21-30	19 (50.0)	
31-40	3 (7.9)	
41-50	2 (5.3)	
> 50	1 (2.6)	
Collection site		
Fixed site	15 (39.5)	0.207
Mobile site	23 (60.5)	
Donor type		
First-time donors	24 (63.2)	0.110
Repeat donors	14 (36.8)	

Of 989 donations tested for antibodies and/or antigens associated with HIV, HBV and HCV infection, 865 (87.46%) donations were detected seronegative versus 124 (12.54%) seropositive cases. HIV infection was positive in 2.53% (25/989) of blood donors, HBV in 7.28% (72/989) and HCV in 2.73% (27/989) of cases. HIV/HBV, HBV/HCV, HIV/HCV and HIV/HBV/HCV coinfections were 0.4% (4/989), 0.71% (7/989), 0.2% (2/989) and 0.1% (1/989) respectively. The seroprevalence of transfusion-transmissible infections was higher among first-time donors and mobile collection sites (**Table 2**).

Prevalence of HIV, HBV, and HCV genomes (DNA, RNA) in seronegative screened blood donors by the CMIA method. Forty-three (43) mini-pools were detected positive for HBV out of the 101 mini-pools of 8 plasma samples tested at the first real-time multiplex PCR. No mini-pool was positive for HIV and HCV. Of 86 mini-pools of 4 plasma samples reconstituted for the second multiplex PCR, 42 were positive for HBV. No mini-pool was positive for HIV and HCV. Of the 168 (42 x 4) samples tested by single PCR, 38 were positive for HBV. Altogether, 4.70% (38/808) of seronegative donations were positive by multiplex PCR. These positive samples were confirmed using the same detection kit on the same device.

Discussion. The present study which aimed to determine the impact of multiplex PCR in reducing the residual risk of transfusion-transmitted viral infections, reported a prevalence of 4.70% (38/808) of HBV viral DNA in seronegative blood donors. Although the sample size is limited, this study is the first using multiplex PCR for the seronegative-blood donors screening in Burkina Faso.

The donations came exclusively from voluntary and unpaid blood donors. In recent years, family or replacement donations have been phased out by the CRTS/O in order to comply with WHO recommendations[11] for better blood safety. Nevertheless, our study reported seroprevalences of 2.53%, 7.28%, and 2.73% respectively for HIV, HBV, and HCV. The seroprevalence of 2.53% of HIV reported in this study is similar to that of 2.00% reported in the general population of the central region of Burkina Faso in 2016[15] where our study was conducted and it is markedly higher than the 0.8% reported in the general population of Burkina Faso by the same study.[15] It is also higher than the 1.8% reported in blood donors from three regional centers in Burkina Faso in 2009[4] but comparable to the 2.21% reported in Koudougou blood donors in 2012.[5] As for HBV, the prevalence of 7.28% obtained in our study was lower than those found in previous studies in the general population[9] and blood donors in Nouna,[7] Ouagadougou[9] and Koudougou[5] or in specific

groups (pregnant women[7] and health workers[6]). The prevalence of 2.73% of HCV infection was also lower than the 4.4% prevalence reported among blood donors in 2014.[8] Altogether, the results of this study show a high prevalence of HIV and HCV against a low prevalence of HBV compared to previous studies in Burkina Faso. This could be explained by the low rate (only 11.3%) of regular blood donor in Burkina Faso.[4]

This decrease in HBV prevalence can be attributed to extensive awareness and screening campaigns during the last decade, significant improvement in the accessibility and availability of hepatitis B vaccine and expanded immunization program against HBV for children at 8 weeks after birth since 2006. In addition, awareness campaigns, which are much more focused on hepatitis B infection with free screening in recent years, are increasingly providing a selective population of voluntary donors at fixed sites who already know their negative serological status for hepatitis B infection but generally unaware of their serology for HIV and/or HCV. This could also explain the high seroprevalence of the latter viruses and low prevalence of HBV infection compared to previous data in Burkina Faso. Furthermore, unpublished data have shown a variation in the prevalence of HBV infection between different districts of Ouagadougou. This observation could be extended to HIV and/or HCV infections. In this study, the seroprevalences of these three viruses were higher in first-time donors than in repeat donors. A similar observation has been made in several studies carried out in Burkina Faso[4] and other West African countries.[16,17] The high prevalence of these viral infections in first-time donors can be attributed to the lower level of knowledge about blood-borne infections and routes of transmission compared to regular or repeat donors. However, to cope with the high demand for labile blood products (LBP), mobile blood collections by CRTS/O is required in high schools, universities, barracks, places of worship etc. These mobile collections would encourage the enrollment of first-time donors although the latter, unlike regular donors, are not always aware of the issue of transfusion safety.[18] This increases the risk of collection of infected donors during seroconversion period. In the present study, 63.90% of blood donors come from mobile sites explaining the predominance of first-time donors (55.21%).

Likewise, the seroprevalences of these three infections were higher in the blood donors at the mobile sites compared to those taken at the fixed site at the CRTS/O with a statistically significant difference ($p < 0.001$) for all these three viral infections. This observation suggests that our method of medical donor selection is not adapted to our blood donors' recruitment policy in mobile sites. Indeed, in mobile sites, blood collections are often organized without prior awareness-raising of potential donors on transfusion safety issues. This is especially true as mobile collections tend to encourage the recruitment of first-time donors. All of these results show that, despite the medical selection of blood donors and the computerization of their data, seroprevalences of HIV, HBV and HCV remain very high among blood donors in Burkina Faso. This reflects shortcomings in the promotion of blood donation and donor education prior to blood collection hence the low proportion of regular donors. These high seroprevalences of HIV, HBV and HCV in a population of volunteer donors also pose the problem of the quality of medical selection. Indeed, a well-conducted medical selection with relevant selection criteria is highly effective in controlling the risk associated with the silent period in infections.[19-21] In Burkina Faso, the pre-donation questionnaire and the quality of the selection have not yet been formally evaluated. Nevertheless, a study by Kafando et *al.* showed that there was no significant difference between the positivity rate among donors accepted to donate and those who were refused.[22]

Of 808 seronegative HIV, HBV, and HCV donations tested by multiplex PCR, 38 (4.70%) residual cases were detected positive. These results are higher than those obtained in similar studies in other developing countries also experiencing high prevalence of these three infections. For example: in Ghana, out of 9372 seronegative screened donors by rapid tests, 3% were detected HBV-positive DNA; no cases of HIV and HCV were detected.[23]

In South Africa,[24] the residual risk rate of HIV, HBV and HCV was estimated to be 1 per 45,765, 1 per 11, 810 and 1 per 732, 200; in Thailand,[25] out of 4798 HIV-negative donations, 6 cases of HBV-positive cases were detected, no HCV and HIV cases were detected. Our results are considerably higher compared to those obtained in developed countries where the prevalence of these three infections is low. For example: United

States[14] (HIV: 1 / 2million, HCV: 1 / 270,000), Italy[26] (HCV: 2.5 / million, HIV: 1.8 / million, HBV: 57.8 / million), Germany[27] (HCV: 1 / 10.8 million, HBV: 1/360 000). All 38 donations tested positive by multiplex PCR were only positive for HBV DNA.

These results suggest that the residual risk of transmission of HBV by blood transfusion is greater compared to that of HIV and HCV in Burkina Faso. Otherwise, the contribution of PCR would be more important for HBV compared to HIV and HCV in our country. The proportion of positive HBV donations that are only detectable in the PCR (4.72%) obtained in our study can be explained by the high prevalence and incidence of this infection in the blood donor population in Burkina Faso.[4] These high prevalences and incidences are necessarily accompanied by significant proportions of infections in the early phase from the point of view of serological markers. Added to this is the high prevalence of occult hepatitis B reported in several studies in Burkina Faso.[28,29] Occult hepatitis B is characterized by the presence of HBV DNA in the serum of a patient who is screened for HBsAg by the usual serological tests.[30] Although our study did not allow us to detect residual cases associated with HIV and HCV, the number of donations detected positive HBV by PCR confirms that PCR is more sensitive than ELISA. This sensitivity had already been demonstrated by some studies conducted in blood donors in Burkina Faso,[31] and in two neighboring countries, Togo[32] and Ghana.[23] The absence of positive cases of HIV and HCV in our study could be explained by the relatively low prevalence of these viruses in the general population but also the limited size of our sample. The residual risk of HIV estimated by a mathematical model based on serological tests was 1 for 55,000 donations among blood donors in Burkina Faso[33] in 2011. Similarly, no cases of residual HIV and HCV risk detectable by PCR were reported in two other similar blood donor studies in Ghana,[23] a neighboring country in Burkina Faso and Kenya,[34] a country of Sub-Saharan Africa like ours. However, cases of residual risk of HIV and HCV, although relatively low compared to HBV, have been observed in similar large-sample studies in other developing countries and developed countries.[13,26,27]

Of the 38 cases of HBV DNA detected in our study, 24 (63.2%) were first-time donors versus 14 (36.8%) who were repeat donors. These results show that the residual risk of transfusion is higher in first-time donors compared to repeat donors, but without a significant statistical difference. These results confirm are consistent with the literature that donations from regular donors would be the most safety. This is a cornerstone of WHO's strategy of promoting regular donations. Nevertheless, we note in our study that residual cases of HBV are also important in repeat donors. From a mathematical model, Nagalo et *al.* estimated equally high incidence rates of 3270.2, 5874.1, and 6784.6 per 100,000 donations for HIV-1, HBV, and HCV, respectively, among repeat donors.[4] Considering a 100% HBV transmission rate by transfusion of a contaminated LBP, the PCR prevented 38 cases of HBV transmission or even more if these 38 donations were used for the preparation of other LBP such as Frozen Fresh Plasmas (FFPs) and Standard Platelet Concentrates (SPC). All of these results show that in addition to promoting unpaid and regular voluntary donation, the implementation of the PCR is useful in the context of Burkina Faso.

Conclusion. The study reported very high seroprevalences of HIV, HBV, and HCV in the blood donor population in Burkina Faso. Multiplex PCR has shown the existence of a high rate of residual cases of HBV associated with ELISA serological tests, which is a serious concern to transfusion safety in Burkina Faso. It is imperative for the CRTS/O to adopt a new screening strategy for blood donations including the Screening of viral genomes of HIV, HBV and HCV for optimal transfusion safety.

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