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Full Length Research Paper

Prevalence of hepatitis virus delta infection among HBVAgs positive blood donors at the Regional Blood Transfusion Center of Ouagadougou, Burkina Faso

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Background: Infection with hepatitis viruses constitutes a public health problem in Burkina Faso. Several previous studies have estimated the prevalence of viral hepatitis A, B and C. This study aimed to study the prevalence of hepatitis delta infection in apparent healthy blood donors positive for the antigen of hepatitis B virus.
Methods: This study involved first time blood donors, positive for HBV surface antigen (HBsAg). The presence of HBV and HDV was confirmed using real time PCR duplex. The SPSS software was used for statistical analyzes of the obtained results.
Results: Two hundred HBsAg positive blood donors were enrolled in this study. The socio-demographic data showed 79% of men from all religious and ethnic denominations. RT-PCR analysis confirmed the presence of HBV DNA in 195/200 (97.5%) positive blood donors. Only 0.5% (1/200) of HBV positive person presented a case of co-infection with HDV.
Conclusion: Our study shows that 97.5% of HBsAg positive person detected by the ARCHITECT i 1000 SRTM were positive by PCR. The obtained results showed that the distribution of HDV in blood donors positive for the hepatitis B antigen is low (0.5%).

Key words: HBV, HDV, Real Time PCR, Blood donors, Ouagadougou, Burkina Faso.

INTRODUCTION

Viral hepatitis, the most widespread human virus infections in the world, is a major public health problem, especially in developing countries (WHO, 2016). A report by the 67th World Health Assembly notes that viral hepatitis is now responsible for 1.4 million deaths a year. About 500 million people currently suffer from viral

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hepatitis and 2 billion have been infected with the hepatitis B virus (WHO, 2016). Most people with chronic hepatitis B or C are unaware that they are infected and present serious risk of cirrhosis or liver cancer (WHO, 2016).

Today, there are an estimated 240 million chronic carriers of hepatitis B virus (HBV), of which about 15-20 million are co-infected with hepatitis D virus (HDV), also called delta hepatitis virus (Blanchet et al., 2014).

HDV is a small, defective circular RNA virus that requires the presence of HBV for its life cycle. This is why it is often called satellite virus of HBV. About 5% of HBV carriers are co-infected with HDV (Ghamari et al., 2013). In combination with hepatitis B, hepatitis delta causes the most severe form of viral hepatitis in humans, including fulminant hepatitis and liver failure, with rapid progression to liver cirrhosis (Alvarado-Mora et al., 2013) followed by hepatic decompensation, and an increased risk of hepatocellular carcinoma (HCC) (Alvarado-Mora et al., 2013).

In Ouagadougou, Burkina Faso, previous studies have shown that HBV seroprevalence was 29.4% in pregnant women (Zeba et al., 2014) compared to 16.59% in blood donors (Nagalo et al., 2012). A study on HDV seroprevalence in a population of blood donors carrying HBsAg in Bobo-Dioulasso, Burkina Faso showed that 3.38% (6/177) were carriers of anti-HDV antibodies (Sawadogo et al., 2016). However, no available study on the prevalence of HDV in the capital Ouagadougou had been published, despite the much higher number of the population in this city. In view of this, there is need for recognizing the safe blood supply to recipients (WHO resolution WHA28.72). Therefore, it is necessary to have reliable data on the seroprevalence of VHD in the city in view to contribute to a better policy for care of this infection. The detection of RNA of the Delta hepatitis virus and especially the quantification of the viral load of the HBV by RT-PCR in real time constitute the main tool for the diagnosis and the management of the infected patients. The presence of Delta RNA makes it possible not only to confirm the replicative nature of the infection, but also to measure the effectiveness of the treatment (Castelnau et al., 2006).

The objective of our study was to determine the seroprevalence of hepatitis delta virus in blood donors positive for HBsAg in Ouagadougou and to specify for the first time the rate of detection of the HDV RNA by real-time PCR.

METHODS

Study population

This was a cross-sectional study conducted from January to September 2015. It focused on a population of first-donors and regular positive HBVAg -donors. They were first tested by an automated serological analyzer (ARCHITECT i 1000 SRTM, Abbott) and by Rapid Testing (HEALGEN China One Step Rapid Test). The socio-demographic characteristics and the anti-HIV and anti-HCV serological results of the donors were collected using questionnaires.

Sampling

Five mL of whole blood was collected from HBV positive blood donors by serological tests performed on the ARCHITECT automaton. After centrifugation at 4000 g for 5 minutes, the plasma samples were aliquoted within 6 hours (h) after sampling.

All the plasma was then subjected to the Diagnostic Rapid Test (DRT). Only samples positive for the ARCHITECT i 1000 SRTM and DRT were selected for the study. The plasma samples were collected in well-identified cryotubes (two cryotubes per sample). The cryotubes were then stored in a cooler during transportation from Regional Blood Transfusion Center of Ouagadougou) to CERBA / LABIOGENE and kept at -80° C till molecular tests.

Detection of HBV DNA and VHD RNA

Extraction of DNA/RNA from HBV / HDV (ref K-2-1/100 Lot 10F15K53)

It was carried out using the Ribo Sorb DNA/RNA kit from Sacace biotechnologies (Como, Italy) (ref K-2-1/100, Lot: 10F15K53) according to the protocol provided by the manufacturer. An internal extraction control was used. The choice of this kit is justified by its capacity of simultaneous extraction of DNA and RNA.

Amplification of HBV/DNA, HDV/RNA (ref V56-100FRT, Lot: 01G15D560)

The principle of real-time duplex PCR HBV/HDV of Sacace includes a step of reverse transcription of HDV RNA followed by real-time amplification of cDNA (HDV) / DNA (HBV). PCR was performed on the SaCycler-96 Real Time PCR System (Sacace Biotechnologies, Italy). The amplification was carried out in a total reaction volume of 25 µL of which 15 µL of Mix and 10 µL of DNA.

The Mix consists of 10 µL of RT-PCR-mix-1 HDV, 5 µL of RT-PCR-mix-2, 0.5 µL of TaqF polymerase, 0.25 µL of RT-G-mix-2 and 0.25 µL of MM1v for each sample. The amplification program consists of 50 ° C for 15 minutes for the reverse transcription followed by 95° C for 15 minutes for the activation of Taq polymerase and then 5 cycles of 95 ° C for 5 seconds at 60° C.

For 25 seconds and 72° C for 15 seconds and finally 40

cycles of 95° C for 10 seconds, 60° C for 30 seconds and 72° C for 15 seconds during which fluorescence is detected. The internal control amplification product was detected on the *Fam* channel with blue fluorescence; that of the HBV DNA was detected on JOE HEX/Cy3 with a green fluorescence while the amplification product of the complementary DNA of the VHD was recorded on Rox/TexasRed with a yellow florescence.

Ethical considerations

This study was approved by CERBA's Institutional Ethics Committee. In conjunction with the Regional Blood Transfusion Center of Ouagadougou administration, we asked each donor for written informed consent for his or her participation in the study. At the end of their interview following post-donation results, a presentation of the present study was carried out. After giving their consent, the blood samples were taken on EDTA tubes identified by an anonymous sampling number, which corresponded to the barcode number assigned by the RBTC / Ouagadougou. Information on each donor enrolled in this study while maintaining anonymity has been listed in an individual collection form.

A second tabular form has been drawn up to collect all the information collected from the individual cards. This second form allows not only to have a duplicate of individual records but also to have an overview at a glance of the collected information.

HEALGEN China One Step Rapid Test

The HBsAg One step Rapid Test Cassette is a lateral flow chromatographic immunoassay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the test. During testing, Hepatitis B Surface Antigen in the serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that the proper volume of specimen has been added and membrane wicking has occurred. When the colored lines are appear, the results are read in 15 minutes.

Automated serological analyzer (ARCHITECT i 1000 SRTM, Abbott)

The ARCHITECT HbsAg Qualitative II assay is a one-step immunoassay for the qualitative detection of HbsAg in human serum and plasma using CMIA technology, with flexible assay protocols, referred to as Chemiflex). In the ARCHITECT HbsAg Qualitative II assay, sample, anti-HBs coated paramagnetic microparticles and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HbsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added to the reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HbsAg in the sample and the RLUs detected by the ARCHUTECT I System optics.

The presence or absence of HbsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HbsAg.

Statistical analysis

The data entry and analysis was carried out using the SPSS software (Statistical Package for the Social Sciences) version 20.0 (SPSS, Chicago, IL, USA). The chi- square test was used for comparison and a P value was significant when <0.05.

RESULTS

Prevalence of HBsAg in blood donors of the RBTC, Ouagadougou

A total of 17,667 blood samples was collected from blood donors and were screened for HBsAg. Of these, 794 samples were tested positive showing a prevalence of 4.5%. Among the 794 samples corresponding to 794 blood donors, at least 400 persons came back for their post-donation serological tests results. The prevalence of delta hepatitis virus was studied among these donors. There were 250 volunteers who met the conditions for inclusion in our study. Of the 250 volunteers, 200 were finally selected because of the availability of the reagents in the Lab (DNA / RNA Ribo Sorb from Sacace biotechnologies (Como, Italy) for real-time RT-PCR Thesocio-demographic characteristics of the study population is presented in table 1. There were 42 women (21%) and 158 men (79%) without any particular ethnic or religious considerations. The majority were within the age group between 19 to 29 years old, or 74% (142/190) of the blood donors in our study. The majority of them (188/200) resided in the city of Ouagadougou.The results show a higher rate of HBsAgs among the youngest (76%)

consisting mainly of students (95/200) ie 47.5% and schoolchildren (50/200) or 25% (Table 2).

The frequency of co-infections among blood donors

HIV and HCV seroprevalences were 1% and 5%; respectively. co-infection was identified in 10 cases including 2 HBV/HIV and 8 HBV/HCV. Furthermore, one of these 10 cases showed to be co-infected with Hepatitis B/Syphilis.

3.3 Prevalence of HBV infection according to blood group (**Table 3):**

The prevalence of HBV infections according to blood group shows a high prevalence of hepatitis B infection in group B (31.5%), and group O + (30.5%) donors.

Results of dual HBV / HDV PCR in blood donors (**Table 4)**

PCR analysis confirmed in 194 HBVAg-positive donors the presence of HBV DNA against 06 donors in whom no HBV DNA or HDV RNA was found. On the other hand, only one case revealed the presence of the RNA of the hepatitis delta virus on the 200 samples analyzed with PCR, ie a rate of 0.5%.

DISCUSSION

The objective of this study was to determine the seroprevalence of hepatitis delta in HBsAg-positive blood donors at the Ouagadougou Regional Blood Transfusion Center and to specify for the first time in Burkina Faso the detection rate of HDV RNA by real-time PCR in this population. We found that the prevalence of hepatitis B in blood donors was 4.5%. This rate is relatively low compared to the rates reported by Nagalo et al*. (2012) and Tao et al. (2014)* who found a rate of 16.59% in 2012 and 14.5% in 2014; respectively. This difference is explained by the fact that regular donors who know their serological status take the necessary precautions to remain healthy.

However, in spite of sensitization campaigns against sexually transmitted infections, young people remain the most infected with HBV. This is confirmed in the study of Tao et al. (2014) who found a prevalence of 19.5% among students. This difference with the results of our study resides in the fact that our study population was all HBsAg+ and predominantly young. In addition, students are more accessible and more inclined to understand and respond to messages from blood donation awareness campaigns. The target population in the study of Tao et al. (2014) concerned anyone wishing to know his result of hepatitis B test and on the other hand included 995 persons against 200 in our study.

The socio-demographic distribution showed that men accounted for 79% of all blood donors. This discrepancy can be explained by the fact that, according to the selection criteria for blood donors at RBTC, Ouagadougou many women find themselves unable to donate blood for reasons such as period of menstruation, pregnancy, the breastfeeding status. The age group 18-29 years represented 76.5% of the study population.

This can be explained by the fact that RBTC, Ouagadougou, according to its own statistics, carries out at least 70% of its blood samplings in secondary schools and universities.

Our study shows a high rate of carriers of HBsAg in young people, particularly students and pupils, respectively 47.5% and 25.0%. The difference between student and schoolchildren rates is explained by the fact that, in addition to being joined in their environment, students go free to donate blood, whereas schoolchildren are only solicited during major campaigns and require with high difficulty authorization from their parents.

According to PCR results, the prevalence of hepatitis delta among donors in our cohort is 0.5% (1/200). Several studies have been conducted in Africa on the seroprevalence of delta hepatitis. These studies for the most part have focused on the search for antibodies to HDV. A study carried out among blood donors of the Regional Blood Transfusion Center of Bobo-Dioulasso using ELISA technique found a seroprevalence of 3.38% (Sawadogo et al., 2016). According to a retrospective study by Andernach et al. (2014) in Bobo-Dioulasso, Burkina Faso, seroprevalence of anti -HDV was 2.5% in 40 mothers compared to 20.5% in 44 children. In Ghana, the prevalence of HDV was 11.3% among 53 people with HBsAg (Asmah et al., 2014).

In Ismalia, Egypt the prevalence of HDV was 4.7% in HBsAg-positive blood donors (Gomaa et al., 2013).Some results of HDV seroprevalence are much higher compared to what we found in our study using real-time PCR. In Tanzania, in a similar study the seroprevalence of HDV was 5%, and no confirmed HDV on 200 HIV/HBV co-infected subjects (Winter et al., 2016). The presence of Hepatitis Delta RNA makes it possible not only to confirm the replicative nature of the infection, but also to measure the effectiveness of the treatment (Castelnau et al., 2006). According to recent studies in Italy, the prevalence of HDV was 8.4%; HDV antibodies were detected in 7.4% of Italians compared with 11.5% in migrants (Rizzetto, 2015).

The cases of co-infection found in our study are 1% for HBV / HIV, 5% for HBV / HCV and 0.5% for HBV / RPR. Cases of co-infection are low compared to the results of some African countries. The HIV / HBV / Ab-HDV prevalence was 25 cases out of 62, ie 40.32% in the city of Bissau in Guinea Bissau in 2011 (Honge et al., 2014); 3 cases out of 61 or 4.91% in a study conducted in Dakar, Senegal (Diop-Ndiaye et al., 2008). One of our specific objectives was to confirm by real-time PCR the

Table 1. Distribution of blood donors AgHBs$^+$ according to sex and age.

Characteristics		Donors (AgHBs$^+$)	Percentage (%)
Sex	Female	42	21,0
	Male	158	79.0
	Total	200	100.0
Age Group	18 – 29	152	76.0
	30 – 39	37	18.5
	40 – 49	04	2.0
	50 – 59	07	3.5

Table 2. Distribution of AgHBs$^+$ donors according to sex and occupation.

	Occupation					
	Trader	**Schoolchildren**	**Students**	**Private sector**	**Public sector**	**Total**
Female	1	14	16	10	1	42
Male	10	36	79	29	4	158
Total	11	50	95	39	5	200

Table 3. Distribution of HBsAg$^+$ carriers according to ABO group and occupation.

Socio-professional characteristics							
		Occupation					
		Traders	**School children**	**Students**	**Private sector**	**Public sector**	**Total**
Blood Group	A	04	18	26	08	01	57
	AB	01	03	05	02	00	11
	B	02	15	33	13	02	65
	O	04	14	31	16	2	67
Total		11	50	95	39	5	200

Table 4. Distribution of carriers of HBV and HDV according to occupation.

		Occupation					
		Traders	**School children**	**Students**	**Private sector**	**Public sector**	**Total**
VHB PCR	Negative	0	3	2	0	0	**5**
	Positive	11	47	93	39	5	**195**
Total		11	50	95	39	5	200
VHD PCR	Negative	11	50	95	38	5	199
	Positive	0	0	0	1	0	1
Total		11	50	95	39	5	200

ELISA results of the RBTC, Ouagadougou. Our study confirmed by PCR the presence of HBV DNA in 194 persons out of 200, i.e a rate of 97% of those already revealed positive by the ARCHITECT i 1000 SRTM. This rate seems to us very acceptable in terms of transfusion safety, since no HBV DNA or HDV RNA was found in the remaining 3%.

Conclusion

The prevalence of delta hepatitis in RBTC, Ouagadougou among hepatitis B surface antigen carriers revealed a low prevalence of 0.5%.

The results of this study make it possible to envisage the prospects in particular to extend the study to a

representative population of Burkina Faso and to look for the genotypes present in Burkina Faso by sequencing and phylogenetic analysis.

Of course this prevalence remains low, but attests the fact that this delta hepatitis virus circulates among our populations. We also recommended It is necessary to conduct further studies on larger cohorts to determine the overall prevalence and clinical significance of delta hepatitis in the country.

Conflict of interest

The authors do not declare any conflicts of interest.

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