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HBc antibody seroprevalence in HBs negative antigen blood donors at the Chad National Blood Transfusion Center in N'Djamena

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The aim of this study is to assess the risk of hepatitis B transfusion in negative HBsAg blood donors who test positive for anti HBcAb. From January to December 2019, an observational study of virological markers was carried out with blood donors (family and volunteers) at the National Blood Transfusion Center in N'Djamena. The donors included were also tested negative for the markers (Ab anti HCV, HIVAg/Ab and TPHA) according to standard methods of clinical microbiology. Of the 1106 donors included in this study, we determined a positivity rate of 41% of anti HBcAb. Significant differences were observed between the proportions of donors: Family (77.48%) and volunteers (22.51%), positive for anti HBcAb (41%) and negative (59.13%), male sex (72%) and female (28.09%) with probabilities of 0.01, 0.001 and 0.001, respectively. This survey made it possible to determine an elevated level of HBcAb in the study population and also to determine about ten cases of cirrhotic patients negative for HBsAg and positive for HBcAb. In view of this result, it is recommended to complete in blood donors with negative HBsAg and HBcAb carriers, screening for anti-HBsAb and, if possible, quantify the viral B DNA in order to minimize the risk of residual hepatitis B transmission among these blood donors in Chad.

Key words: Anti-HBc antibodies, seroprevalence, blood donors, hepatitis B, N'Djamena.

INTRODUCTION

The hepatitis B virus (HBV) is one of the leading causes of liver inflammation posing a serious public health problem worldwide (COMEDE, 2012; Meffre et al., 2010).

According to the World Health Organization (WHO), 325 million people worldwide live with chronic infection with the hepatitis B virus (HBV) (Andreu, 2012; Vaux et al.,

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2019).

The prevalence of anti HBc antibodies has been estimated at 7.3% in the general population aged 18 to 80 years in Metropolitan France, which represented 3.5 million people who had been infected with HBV during their lifetime (MDM, 2011). Although vaccination against hepatitis B is an effective preventive measure, infection with hepatitis B virus remains a major public health problem (POC, 2018; Brouard et al., 2013; Syria et al., 2012).

The transmission mode is variable (HAS, 2012; WHO, 2015). In low endemicity areas (Percentage of HBsAg<2% in the population: North America, Australia and Western Europe), transmission is parenteral and sexual. In highly endemicity areas (Percentage of HBsAg>8% in the population: Africa, Southeast Asia, China, Japan), mother-to-child transmission is predominant. In Cameroon, also a highly endemicity country, markers of the hepatitis B virus are found in around 10% of the population (Brouard et al., 2016; Schweitzer et al., 2015; INSPQ, 2016). To date, Chad does not have national HBV seroprevalence data. However, the World Health Organization places Chad in the high endemicity area. An observational study was performed in cirrhotic patients with normal transaminase revealed the absence of HBs Antigen and the presence of anti-HBc Antibody (Registers, 2015-2020). Another study carried out on 2238 volunteers in N'Djamena by Bessimbaye et al. (2014) showed an HBsAg seroprevalence of 13.5% but it did not test for anti-HBc antibodies (Bessimbaye et al., 2014).

The search for HBV markers can be carried out either by enzyme-linked immunosorbent assays (ELISA) detecting direct markers in the blood (HBsAg, HBeAg, viral DNA), or even histological section of the liver (HBe antigen), or markers indirect (anti HBc IgM type antibodies and IgG type antibodies) of the infection, either by molecular tests detecting, quantifying or characterizing the DNA sequence of HBV. The advent of new high-performance tests (PCR RT, chemiluminescence, etc.) for detecting infectious risks on donations has made it possible to considerably reduce the risk of transmission of viral infections through blood products (ASPC, 2020; Larsen et al., 2005; Bottero et al., 2016). With a view to optimizing diagnostic strategies aimed at improving the quality of labile blood products, it is important to identify the serological profile of markers that may make it possible to avoid the risk of this condition in recipients of these products and their long-term consequence (Lefrere and Rouger, 2011; Pillonel et al., 2012).

Systematic screening for HBs Antigen on all donated blood has radically reduced the risk of infection by transfusion of the hepatitis B virus. However, there remains a residual risk of transmission of the hepatitis B virus; this risk could be four factors: a technical error; a viral variant not recognized by certain reagents; a blood

donation from a recently infected subject; an infectious blood donation by a chronic seronegative carrier.

This study aimed to determine the percentage of positivity of anti-HBcAb in HBsAg negative blood donors. Also indicate the most appropriate HBV screening strategy(s) in blood transfusion (PA, 2014; WHO, 2016; Lange et al., 2017).

The results of this work could be a good awareness-raising tool with a view to actively participating in the fight against HBV in the search for maximum safety in the transfusion process and the establishment of a haemovigilance system in Chad.

MATERIALS AND METHODS

Study framework

This is a prospective observational study that took place in N'Djamena (Chad) for the recruitment of blood donors. Blood samples collected from donors for HBV markers were tested:

- (1) At the laboratory of the National Blood Transfusion Center (CNTS) in N'Djamena (Chad);
- (2) Laboratory Immunology Unit of the National Reference University Hospital (CHU-RN) of N'Djamena (Chad);
- (3) Center for Study and Research in Applied Biology (CERBA) of Paris/France, within the framework of the agreements for the execution of examinations impossible to carry out on site, as for the quality control and evaluation of our results where all the electrochemiluminescence diagnostic steps were performed.

Selection of blood donors

The biological test strategy for screening donations blood for HBV can be summarized as four markers of transmissible diseases, one of which is bacterial (syphilis) and three viral (hepatitis B and C viruses, human immunodeficiency virus (HIV)). Blood donors were tested either by an Immunoserological test system with VIDAS or by Immunochromatographic tests for the detection of Ag/Ab.

Inclusion and exclusion criteria

Included were any blood donors who tested negative for the markers: HBsAg, HCV Ab, HIV Ag/Ab, and TPHA.

Not included were blood donors with clinical anemia, pregnant, postpartum or breastfeeding women, people with chronic pathology and subjects who tested positive for at least one of the markers: Ag HBs, HCVAb, Ag/Ab HIV, and TPHA.

The donors recruited at the CNTS were family donors and unpaid volunteers subject to residual risk of high contamination.

Family donors or replacement donors: Donors providing assistance to a sick relative;

Volunteers donors: Donors intervening in the context of assistance to the person in danger.

Sample collection

An anti-HBc antibody research was performed in 1218 informed consent negative HBsAg blood donors. In total, 1106 HBsAg blood donors were selected for the study. This is a population of

voluntary donors, aged at least 18, from all professions and social categories. The serum or plasma was collected from January to December 2019 in the CNTS laboratory either on EDTA-impregnated tubes or from bags of blood impregnated with citrate in preparation after separation of the plasma and the red blood cell concentrate. After centrifugation at 40,000 g for 5 mn, the serum was collected in 1.8 ml cryotubes and stored at -20°C.

Certain parameters were also collected on donors such as: sex, age, marital status, profession, type of donation, risky behaviors (previous transfusion, surgeries, multiple sexual partners, etc.), and the system used for serological screening, serological status, place of blood donation, etc. Analysis of these parameters has provided a better understanding of the profile of blood donors facing HBV infection and the risks of transmission of other viral diseases transmissible through blood transfusion.

Data processing

Data were entered and analyzed using Microsoft Word and Excel 2013. The Chi-square test (χ^2) was used to compare the qualitative variables with a significance level set at 5%.

Microbiological analysis

Presentation tests

Organic products reviewed were serum or plasma. These products have been tested in laboratories with the various screening kits and we have taken the electrochemiluminescence performed by the CERBA COBAS machine as a reference method.

Qualitative determination of total anti-HBc antibodies and HBsAg by the VIDAS machine (BioMérieux)

The BioMérieux VIDAS robot system was used for the detection of antigen-HBs and total anti-HBc antibodies (HBcT). A positive and negative quality control system for each run is available to validate a system test kit and internal control for each sample unit use. Barcodes ready to use reagents (reconstitution for some) were used.

The software supplied with the VIDAS system includes programs for analysis and data management. A two-way computer interface automatically transfers results to the user's Laboratory Information System (LIS) and to various product and patient reports. This avoids human errors in reading the results. A quality control system is available to validate a VIDAS system test kit.

As part of our work, VIDAS HBcT and HBsAg cartridges (BioMérieux) were used for the detection of anti-HBcT Ab and HBs Antigen. The VIDAS has 5 compartments. After collection of whole blood in a dry tube or lithium tube and centrifugation, 150 μ l of serum was collected and transferred to the first well of the VIDAS HBcT cartridge and HBsAg. The cartridge is placed in the corresponding compartment of the VIDAS. The automaton is started by clicking on the appropriate compartment and the test result is expected within 1 h and 21 min.

Qualitative determination of total anti-HBc antibodies by the COBAS robot system (CERBA)

The immunological test for the qualitative determination of total anti-HBc antibodies is carried out with human serum or plasma. This electrochemiluminescence assay (ECLIA) is used on COBAS immunoassay systems (Roche, Germany).

COBAS systems have powerful software including data analysis and management programs. A positive and negative quality control system for each run is available to validate a system test kit and internal control for each sample. Single-use, barcode, ready-to-use reagents (no freezing or reconstitution) was used.

The principle of COBAS is based on the pretreatment incubation of 40 μ l of sample with a reducing agent. Then the HBcAg is added and incubated at 20 to 25°C. An immune complex is formed with the HBc antibodies in the sample. Biotinylated Antibodies, ruthenium labeled HBcAg specific antibodies and streptavidin coated microparticles were added and incubated. The complex has just become fixed on available sites of HBc antigens. The complex is attached to the solid phase by a biotin-streptavidin bond. The reaction mixture is transferred to the measuring cell, the microparticles are held at the level of the electrode by a magnet. Removal of the free fraction is accomplished by passing ProCell or ProCell M. A potential difference applied to the electrode triggers the production of luminescence which is measured by a photomultiplier. The software automatically determines the results by comparing the electrochemistry luminescence signal generated by the reaction with the cutoff value that was obtained during a calibration. The CERBA results are returned to us as part of the collaboration of subcontractors between CERBA and the N'Djamena CHU-RN laboratory for the comparison of the two methods used for the analysis of the samples.

RESULTS

Study population

In total, 1218 blood donors were recruited. Among these, 112 blood donors with clinical anemia, pregnant, postpartum or breastfeeding women, people with HBV vaccination status or a chronic pathology and subjects who tested positive for at least one of the markers: HBs Ag, anti-HCV Ab, Ag/Ab-HIV and TPHA.

In total, we selected a population of 1106 HBsAg negative donors who were collected. The average age of the 1106 donors was 39 years with the extremes of 18 and 60. The 18 to 28 age group was the most represented with (521/1106) or a proportion of 47.10% of donors (Figure 1).

Blood samples (1050) were analyzed with the VIDAS machine at the immunology laboratory of the National Reference University Hospital (CHU-RN) of N'Djamena to search for HBcAb and 56 were selected at random and sent to the Research Center in Applied Biology (CERBA) from Paris, France to be analyzed by the electrochemiluminescence method. Indeed, the size of the samples sent to CERBA was small but the results obtained from these samples were used as quality control to evaluate the results obtained at the laboratory of the CHU-RN of N'Djamena.

Family donors were the most numerous 857 (77.48%), against 249 (22.51%) of voluntary donors ($p = 0.01$, significant difference). Female donors were 52.53% (581/1106) and 47.46% (525/1106) donors were male ($p = 0.50$, not significant difference). The male/female sex ratio in the present study is 1.11 (581/525) (Table 1). The donors were divided into categories according to the

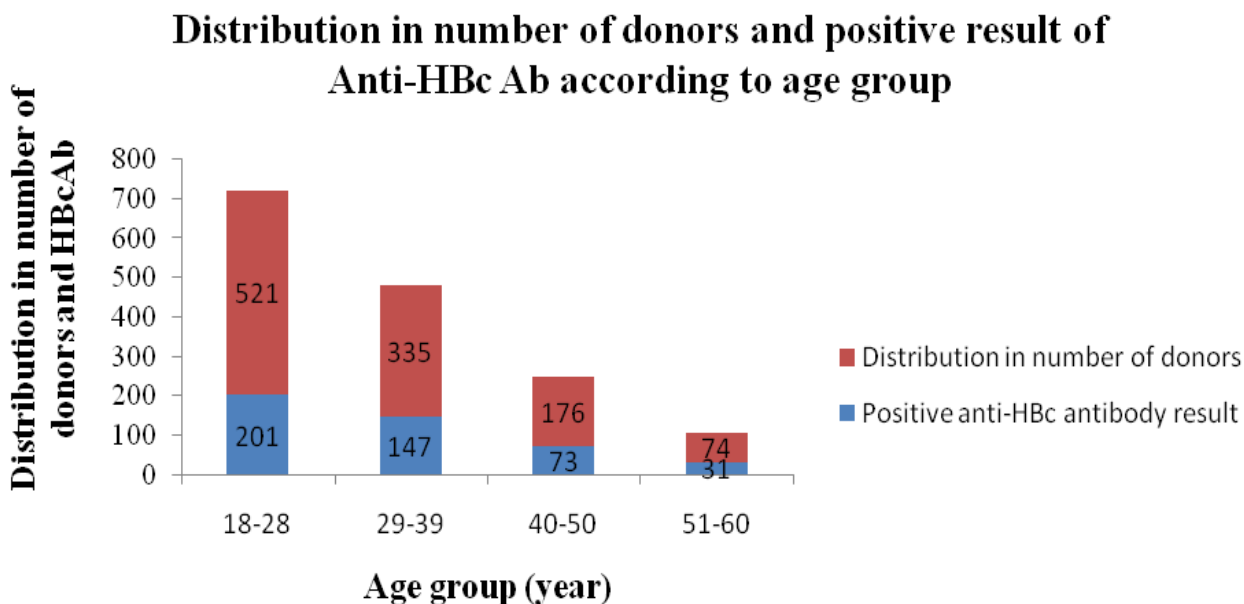


Figure 1. Distribution of 1106 donors and 452 positive results for anti-HBc Ab according to age group.

place of donation: MILITARY (all dressed bodies); SCHOOLS (pupils and students of universities and large schools); ASSOCIATION (Red Cross, footballers, functionary and company workers, etc.); CHURCH (places of worship: Protestants, Catholics...); MOSQUE (places of worship: Muslims...); CNTS (fixed collection at the national blood transfusion center: all professions and social categories combined).

Distribution of donors according to location of donation, types of donors, and positive HBc antibody results

The distribution of the results according to the place of donation gave for the positive anti HBcAb status of the donors: 61.53% (5/13) for the ARMY, 35.58% (305/857) at the CNTS, the ASSOCIATIONS 62% (40/64), SCHOOLS 44.23% (23/52), CHURCH 56.7% (34/60) and MOSQUE 70% (42/60), respectively (Table 1).

Analyses of 1050 samples from HBsAg negative donors carried out in Chad (Table 1), gave 417 samples positive for HBcAb (40%) and 633 (60.28%) were negative for HBcAb ($\chi^2 = 7.216 > \chi^2_{0.05} > 3.84$, $p = 0.01$, dof = 1, significant difference).

In contrast, of the 56 specimens sent to CERBA in Paris, France, 35 (62.5%) were found positive for HBcAb and 21 (37.5%) were negative for HBcAb.

According to gender, it appears that 294 (28%) positive HBcAb donors were male versus 123 (12%) female donors positive for HBcAb ($\chi^2 = 5.949 > \chi^2_{0.05} > 3.84$, $p = 0.02$, dof = 1, significant difference). The results of the CERBA analyzed in France were 31/49 (63.26%) for

males and 4/7 (57.14%) for females ($\chi^2 = 0.049 < \chi^2_{0.05} < 3.84$, $p = 0.50$, dof = 1, non-significant difference).

The cumulative results of the two laboratories (CHURN, CERBA) revealed 452 HBcAb positive donors (41%) and 654 (59.13%) negative HBcAb donors ($p = 0.001$, significant difference). According to gender, the cumulative results of the two laboratories gave a seropositivity rate of HBcAb of 28.09% in women (127/452) and 72% in men (325/452) ($p = 0.00$, significant difference).

Distribution of samples according to socio-demographic group exposed to residual risk of transfusion and positive HBcAb status

The distribution of positive anti HBcAb was: 37% (198/499) for married, 30% (87/292) single, 45.28%, (48/106) divorced, 62% (90/146) sex workers, and 61.53% (8/13) military, respectively. High levels of positive anti-HBcAb were observed in sex workers and military personnel followed by divorced and celibates (Figure 2).

Distribution of donors and positive results for HBcAb according to profession

The most represented profession was functionary with 251/1106 (23%) donors of whom 94/251 (37.45%) were positive for HBcAb followed by 133/1106 housewives (12.02%), 112/1106 resourceful (10.12%), 105/1106 pupils (9.49%) and 102/1106 traders (9.22%) including 77/133 (58%), 54/112 (48.21%), 41/105 (39.04%) and

Table 1. Distribution of donors according to sex, types of donors, place of donation and status anti-HBcAb.

Parameter	Place of donation						Total (%)
	Church (%)	Mosque (%)	CNTS (%)	Association (%)	Establishment school (%)	Military camp (%)	
Sex							
Male (%)	30 (50)	60 (100)	362 (42.24)	40 (62.5)	20 (38.46)	13 (100)	525 (47.46)
Female (%)	30 (50)	0 (0)	495 (58)	24 (37.6)	32 (61.53)	0 (100)	581 (52.53)
Total	60	60	857	64	52	13	1106 (100)
Types of donors							
Volunteers (%)	29 (48.3)	14 (23.3)	190 (21.17)	8 (12.5)	6 (11.53)	2 (15.38)	249 (22.51)
Family (%)	31 (51.7)	46 (76.7)	667 (78)	56 (87.5)	46 (84.46)	11(85)	857 (77.48)
Total	60	60	857	64	52	13	1106 (100)
Status anti-HBcAb							
Négative (%)	26 (43.3)	18 (30)	552 (64.41)	24 (37.5)	29 (56)	5 (37.46)	654 (59.13)
Positive (%)	34 (56.7)	42 (70)	305 (35.58)	40 (62.5)	23 (44.23)	8 (61.53)	452 (41)
Total	60	60	857	64	52	13	1106 (100)

63/102 (62%) were positive for HBcAb, respectively (Figure 3).

Distribution of donors and anti-HBcAb positive results according to age group

The distribution by age group showed a predominance in the age group of 18 to 28 years with 521/1106 (47.10%) of donors of which 201/521 were positive for HBcAb (38.57%) followed by those aged 29 to 39, 40 to 50, 51 to 60 years with 335/1106 (30.28%), 176/1106 (14.46%), 74/1106 (7%) of donors of whom 147/335 (44%), 73/176 (41.47%) and 31/74 (42%) were positive for HBcAb, respectively (Figure 1).

DISCUSSION

At the end of this work, which focused on the

proportion of anti-HBc antibodies, in HBsAg negative blood donors at the National Blood Transfusion Center (CNTS) of N'Djamena, it appears that 452/1106 HBsAg negative donors were positive for anti-HBcAb (41%). This rate is higher than those obtained by Villar et al. (2011). On the other hand, in terms of the number of cirrhotic patients positive for anti-HBcAb, the results of this study corroborate those of the other authors (Bottero et al., 2016; ECDC, 2018). This study showed a strong participation of female donors. Female donors were 581 (52.53%) and 525 (47.46%) donors were male. The massive participation of women in the study could be explained by the high level of compassion of women towards a sick relative and also by the proportion of women of 52% in Chad (RGPH 2, 2009). Blood safety therefore remains a major public health problem in the world. On the other hand, in terms of the number of cirrhotic patients positive for anti-HBcAb, the results of this study

corroborate those of the other authors (Bottero et al., 2016; ECDC, 2018). According to the PHE, the residual risk is the risk of transmitting a virus through transfusion, despite the measures taken to select donors and screen for biomarkers of viral infection. It is represented almost exclusively by infectious donations, collected during the window of serological silence, which corresponds to the period between contagion and the appearance of the serological marker (antigen or antibody) sought in blood donations (EASL, 2012). The risk of contamination of hepatitis B by transfusion of blood tested for positive anti-HBcAb in a donor of HBsAg negative blood during a transfusion in a context of occult hepatitis is well known (ECDC, 2018; Gkouvatso, 2017). Occult hepatitis B is defined by the presence of HBcAb in the blood of a subject tested for negative HBsAg, and characterized by the presence of HBV DNA in the serum and/or in the liver of a patient whose HBsAg is not detectable by the usual serological

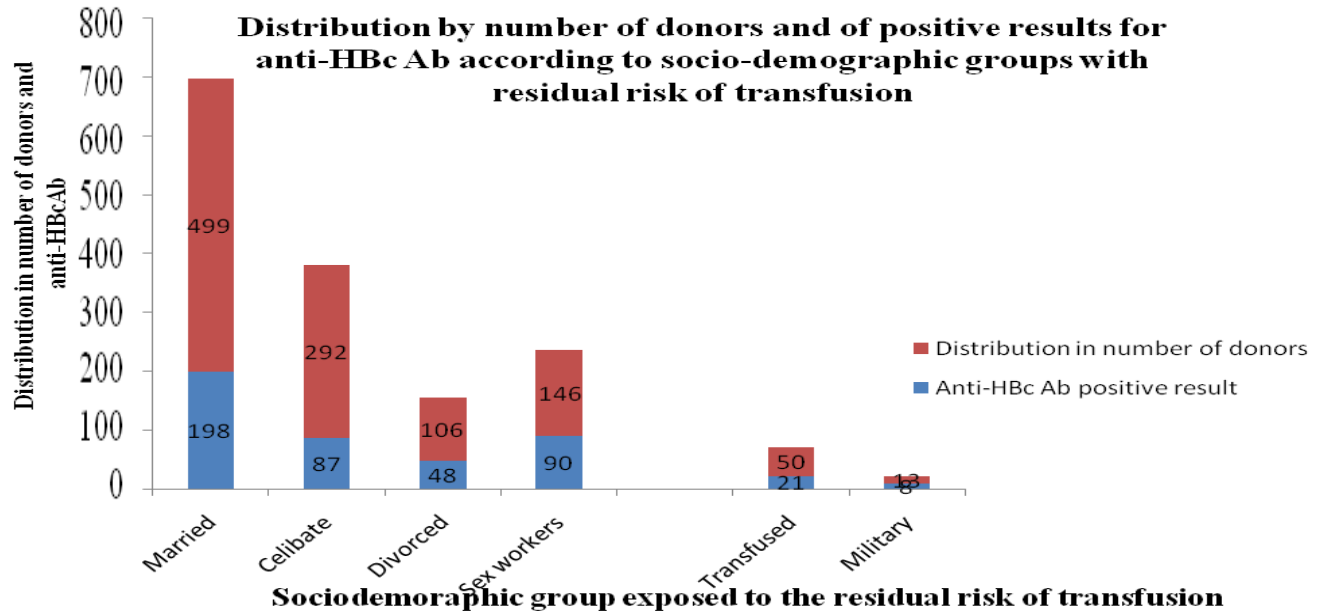


Figure 2. Distribution of 1106 donors and 452 positives HBc Ab results according to the socio-demographic group.

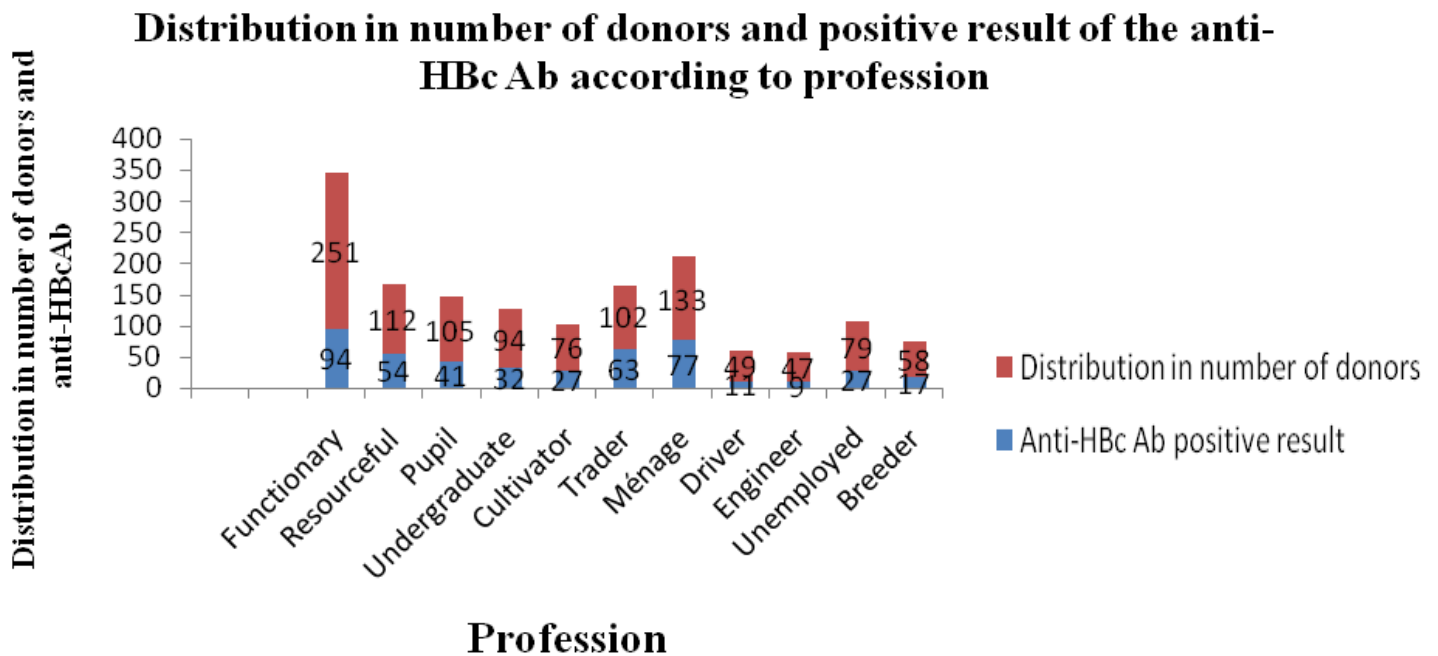


Figure 3. Distribution of 1106 donors and 452 positive results for HBc Ab according to profession status.

tests. In practice, two situations likely to mimic occult hepatitis should be excluded:

(1) In the incubation phase of an HBV infection characterized by an immunological window where HBsAg has not yet become positive, even though viral B DNA is present.

(2) During the healing period characterized by the disappearance of HBsAg, and when the HBV genome is still detectable with the corresponding antibodies (Laperche et al., 2012).

In fact, since Chad is located in an area of high endemicity for the carriage of hepatitis B markers, the problem is of definite interest in terms of blood transfusion

(Dhumeaux et al., 2014).

To do this therefore, it is important to assay the 3 hepatitis B markers: HBsAg, HbCAb, anti-HBsAb before any transfusion decision as recommended (WHO, 2016; Laporal et al., 2019) diagram, only people screened negative for HBsAg and HbCAb can be considered as safe donors since they have never been in contact with the hepatitis B virus. In contact subjects, that is to say with positive HbCAb, it is recommended to take into account the anti-HBsAb as best as possible to supplement with the viral load to know whether they are cured and thus limit the risk of transmission of occult viral hepatitis B (Papatheodoridis et al., 2016; Raffeti et al., 2016; Rahib et al., 2019). In Europe, the major interest in identifying these occult infections lies in the risk of transmission of HBV in hemodialysis patients, during organ or blood donation (Richard et al., 2017; SFM, 2015). In Africa, south of the Sahara, an area of high endemic for hepatitis B, the problem becomes even greater when we know that the blood transfusion systems are not efficient. In addition to the ethical aspects, this is a highly worrying issue falling within the framework of a blood safety policy (Meffre et al., 2010; Michele et al., 2011).

In Chad, data from the CHU-RN gastroenterology department register revealed around ten cases of cirrhotic patients negative HBsAg and positive of HbCAb (Registres, 2015-2020). In routine consultation, the proportion of contact cases is high and is responsible for most chronic negative HBsAg liver disease in our Chadian context. Admittedly, no study was done before even if the finding on the ground is indisputable, this study confirms it.

The seroprevalence of the carriage of anti-HbCAb is superimposed on the geographical distribution of the seroprevalence of markers of viral hepatitis B, and characterized in areas of high endemicity (sub-Saharan); an area of medium endemicity (Mediterranean) and an area of low endemicity (European) (Laperche et al., 2012; PA, 2014; EASL, 2017). In blood donation establishments, any blood product tested positive for HbCAb is excluded from donating blood and the donor is referred to a specialized center for biological monitoring and medical treatment even if international recommendations are not consensual on the management of isolated HbCAb (Michele et al., 2011; WHO, 2016). According to French recommendations, it seems to indicate to assay viral DNA in order to identify cases of occult hepatitis. If the DNA is negative, vaccination can be offered (HAS, 2012; MDM, 2011; Denis and Daddi, 2016; PHE, 2017).

Antibodies to the HBV capsid proteins (anti-HBc antibodies) are the best serological marker of contact with HBV. To this end, the initial search strategy for the detection of HBs Ag should be recommended in our region and in case of negativity, sought for anti-HbCAb and anti-HBsAb.

Anti-HBs antibodies also appear in the serum of patients vaccinated against HBV. In this case, their

presence is not associated with that of anti-HbCAb IgM-type and is present in high titer during acute infection. They may also be present in a low and fluctuating titer during the immuno-elimination phase of chronic hepatitis B or recur with reactivation of chronic hepatitis B in an inactive HBV carrier. In some cases, the presence of anti-HBc antibodies is the only virological marker present in a subject infected with HBV. This situation can be observed:

- (1) During acute hepatitis which follows the disappearance of HBsAg and precedes serological recovery characterized by the appearance of anti HBs antibody; in this case, the isolated presence of anti-HBc IgM and the subsequent appearance of anti-HBs antibodies allow the diagnosis;
- (2) In "cured" patients who have lost their anti-HBs antibodies;
- (3) In patients with occult hepatitis B infection, defined by the presence of HBV DNA in the liver, while HBsAg, produced in very small amounts, is undetectable by conventional commercial tests. In these patients, serum DNA may be detectable (generally <200 IU/ml) or undetectable (Dhumeaux et al., 2014; ECDC, 2018; INSPQ, 2014; Raffetti et al., 2016).

The solution for Chad and the developing countries in areas of high endemics was to first recommend a low-cost screening algorithm which consists in detecting the majority HBsAg and secondarily detecting the anti-HBc antibody and better to supplement with the detection of viral HBV DNA.

Conclusion

Detection of isolated HbCAb remains very high in blood donors who tested negative for HBsAg in Chad. Transfusion safety depends on one hand, through better selection and retention of blood donors, and on the other hand, through the early diagnosis of the main viruses transmissible through the blood that may infect the recipient. The use of more sensitive and specific molecular techniques brings a decrease in the silent window and eliminates false positives, false negatives in the detection of viruses transmitted by blood. It would also be desirable to continue the study of the frequency of anti-HbCAb in blood donors in other regions of Chad, and also to promote vaccination against hepatitis B, especially in children under five years, and depending on gender.

Although localized, this study made it possible to determine a high level of HbCAb in the study population of blood donors and around ten cases of cirrhotic patients negative for HBsAg and positive for HbCAb in the gastroenterology department of the CHU-RN.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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