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Implication Of Isolated Anti-Hbc Antibodies Among Mauritanian Blood Donors

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ABSTRACT

Blood transfusion safety is an important health issue particularly in regions endemic to hepatitis B virus (HBV) infection. In this research, our goal was to investigate the prevalence of isolated HBV core antibodies (IAHBc) in Mauritanian blood donors and the risk of hepatitis B reactivation associated with this serological pattern. Samples of HBV surface antigen (HBsAg)-negative blood donors were collected from the National Center of Blood Transfusion in Nouakchott and screened for both hepatitis B core antibodies (anti-HBc) and hepatitis B surface antibodies (anti-HBs) using Mini VIDAS. Plasma HBV DNA was determined using a quantitative real-time polymerase chain reaction in subjects with isolated anti-HBc (IAHBc). Out of the 320 healthy donors recruited for this study, 48.7% (156/320) had anti-HBc and 14.7% (47/320) were IAHBc carriers. No HBV DNA was detected in any of the subjects. In the first evaluation of blood safety in the country, no occult HBV infection was detected. Nevertheless, screening for IAHBc and anti-HBc antibodies should be introduced, considering the risk associated with these antibodies and their relatively high prevalence in our cohort.

1. INTRODUCTION

It is estimated that more than one million people were infected each year and about 296 million people were chronic carriers of hepatitis B virus (HBV) in 2019 [1]. The natural course of the disease is mainly affected by the interaction between the virus itself and the host [2]. Among other serological markers, different anti-HBV antibodies were used to trace the phases of the infection. While hepatitis B core antibodies (anti-HBc) specify a preceding or ongoing infection, the presence of hepatitis B surface antigen (anti-HBs) antibodies is typically a sign of recovery and considered protective against the disease. An uncommon serological profile consisting of the absence of HBs antigen and the presence of anti-HBc but no anti-HBs antibodies has been reported in numerous studies [3], [4]. This pattern, referred to as "isolated anti-HBc" (IAHBc) [5], may reflect different HBV-correlated conditions such as resolved or chronic hepatitis, occult infection or a false-positive

anti-HBc test result.

The WHO Expanded Program on Immunization (EPI) was established in Mauritania in 2004, nevertheless hepatitis B is still a major health concern in Mauritania, with a global prevalence of HBV surface antigen (HBsAg) exceeding 10% [6], [7]. High exposure rates to HBV have been reported in pregnant women, patients, and healthcare workers [8], [9]. Exhaustive screening for HBV serologic markers (anti-HBc and anti-HBs) among blood donors remained poor, which could largely affect the safety of blood donation as well as the way the disease is treated.

This study aimed to assess the prevalence of anti-HBc antibodies among blood donors in Nouakchott City and discuss the specific implications of the isolated anti-HBc profile.

2. PATIENTS AND METHODS

A total of 320 HBsAg-negative plasma samples were collected from healthy volunteer blood donors at the National Center of Blood Transfusion in Nouakchott.

2.1 Detection antibodies

The subjects enrolled in this study were negative for hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis virus. Serological screening for hepatitis B antibodies was performed using standard laboratory tests. Anti-HBc and anti-HBs antibodies were detected using an enzyme-linked fluorescent assay (ELFA) with an immunoassay analyzer (VIDAS® BIOMÉRIEUX Diagnostics, France), according to the manufacturer's instructions. Reactive samples for anti-HBc and anti-HBs antibodies were re-tested to eliminate false positive results.

2.2 HBV viral load quantification

HBV DNA was extracted using EZ1®DSP Virus Kit (Qiagen) according to the manufacture instructions on the EZ1 Advanced XL platform (QIAgen, Hilden, Germany). The extraction was eluted in a final volume of 60 µl. HBV DNA levels were measured by real-time PCR. A standardized commercial assay was used, the Artus® HBV RG PCR kit (QIAgen, Hilden, Germany) on a Rotor-Gene Q real-time thermal cycler (QIAgen, Hilden, Germany). One negative control and four standards were used with each PCR run. In this essay, the viral DNA detection limit is set to 3.8IU/ml according to user manual.

2.3 Statistical analysis

Cochran–Armitage trend test was performed using SPSS (version 25). Data were summarized and presented as descriptive measures, such as tables, figures, means, and percentages. The 95% confidence interval (95% CI) was calculated.

3. RESULTS

All participants were Mauritanians. Their ages ranged from 18 to 58 years old with a mean age of 31.5 years. Male and female represented 84% (252/320) and 16% (49/320) of the total cohort, respectively (Table 1).

While 48.7% (156/320) of the donors were positive for total anti-HBc antibodies (IgM + IgG), 49.7% (159/320) lacked anti-HBc antibodies (Fig.1).

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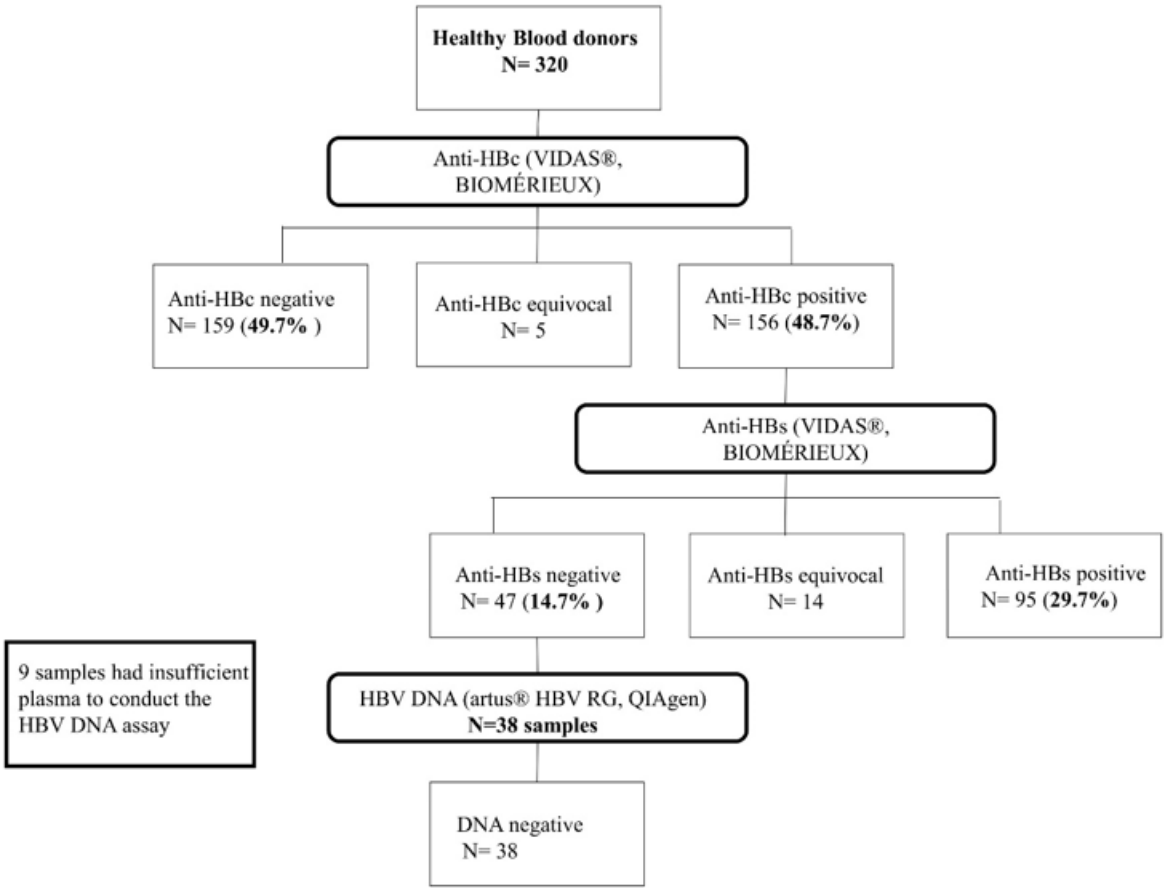

Figure.1: Hepatitis B serological and molecular tests in blood donors

Among the 156 anti-HBc-positive subjects, 29.7% (95/320) had protective anti-HBs antibodies, Isolated HBV antibody (IAHBc) carriers accounted for 14.7% (47/320) of all recruited blood donors (Fig.1).

The distribution of anti-HBc serological patterns showed that isolated-HBc antibodies were more prevalent in older individuals (31-40 and sup à 41, respectively) (Table 1). Additionally, The Cochran–Armitage trend test showed a significant linear trend between the prevalence of anti-HBc and advancing age (z= -7.2865, two sided p<0.0001). In contrast, anti-HBc seronegativity was more frequent in the adolescent and young adult groups (18-20 and 21-30, respectively) (Table 1).

Among the 38/47 samples, where HBV viral load could be quantified, no HBV DNA was detected in any of the IAHBc carriers tested. Samples were retested on the same DNA extract to eliminate the possibility of false-negative results.

Table 1. Pattern of HBV markers and age distribution of blood donors' cohort

Pattern of HBV serological markers	**Anti-HBc (IgM and IgG) negative**	**Anti-HBc (IgM and IgG) positive**	**Total**

		Anti-HBs positive	**Anti-HBs negative (Isolated anti-HBc)**	
Prevalence N (%)	159 (49.7%)	95 (29.7%)	47 (14.7%)	**320**
Gender				
Male	128 (80.5%)	82 (86%)	42 (89%)	**252 (84%)**
Female	31 (19.5%)	13 (14%)	5 (11%)	**49 (16%)**
Age group (Years)				
18-20	43 (27%)	1 (1%)	0	**44(15%)**
21-30	77 (48%)	25 (26.3%)	10 (21.3%)	**112(37%)**
31-40	30 (19%)	47 (49.5%)	19 (40.4%)	**96(32%)**
Sup à 41	9 (6%)	22 (23.2%)	18 (38.3%)	**49(16%)**
Total	**159**	**95**	**47**	**301**

4. DISCUSSION

Isolated anti-HBc (IAHBc) is a specific HBV serological profile in HBsAgs negative subjects concomitantly with the presence of anti-HBc but the absence of anti-HBs antibodies [5]. The percentage of isolated anti-HBc antibodies worldwide varies with the endemicity of the HBV infection. For instance, in areas of low hepatitis B prevalence, the occurrence of IAHBc among blood donors was estimated to fluctuate between 1% to 10 % [3] and increases considerably in populations where the infection is common, as in China (11.9%) [10], Saudi Arabia (18%) [11] or Ghana (up to 40%) [10].The prevalence of IAHBc in our cohort (14.7%) fits the pattern of high HBV incidence reported in the Mauritanian population [6], [7], and also the elevated percentage (48.7%) of donors positive for anti-HBc antibodies in the present study, indicating a previous or ongoing hepatitis B infection.

False-positive anti-HBc due to defective testing assays could be ruled out here, as we used a trustworthy enzyme immunoassay (VIDAS® BIOMÉRIEUX Diagnostics, France) and re-tested all positive plasmas with isolated anti-HBc antibodies.

Although either a transient state (window period) of seroconversion, between the loss of HBsAg and the appearance of anti-HBs antibodies, or an occult HBV infection (OBI) may also underlie this isolated anti-HBc status, we did not detect HBV DNA in any of the 38 samples with IAHBc for which the plasma viral load could be assessed using the reliable real-time PCR test. There have been reports that HBV DNA is either negative [13] or had a very small percentage of positivity in anti-HBc carriers from blood donor samples [14], [15]. For instance, in Cameroon where the overall prevalence of hepatitis B ranges from 16-19.9%, among 522 HBsAg-negative and anti-HBc-reactive patients, only 0.56% had an OBI with a low HBV DNA load of less than 6IU/ml [16].

Therefore, we assumed that the IAHBc in our cohort was more likely a consequence of a past resolved infection, after which anti-HBs normally decreased to an undetectable level due to waning immunity [17], [18]. To maintain a protective level of specific antibodies, the memory B cell model requires regular activation by antigen-specific stimulation, such as booster vaccination and frequent infection, in order to proliferate into long-term antibody-secreting cells [19]. Although fading antibody levels after recovery from a disease may therefore be normal due to the reduction in the number of specific B and T memory cells, the etiologies of such immunological memory decline have been associated with different factors. These events include genetic errors in B-cell development, immune regulation and infections [20- 22]. This deficiency of a persisting antigen that induces B cell inactivation is consistent with the data we found, that is, isolated HBc antibodies were more prevalent in older individuals.

The significance of such an assumption is also supported by numerous studies [16], [23]. Thus, in an ethnically different and geographically remote population, the general prevalence of IAHBc in Korean subjects was found to increase with age (from 0.7% in the ≤ 20 years age group, to 24.2% in the >80 years age group) [4].

The fading of the humoral immune response after recovery is neither new nor specific to hepatitis B. In the reminiscent waves of the COVID-19 pandemic, studies have shown that anti-SARS-CoV-2 antibody levels decline with time [24], [25]. Similarly, a recent study conducted in Mauritania examined the longevity of antibody titers following infant immunization and found a decrease in anti-HBs levels over time [26].

Limitation: Precise occult HBV infection refers to the replication-competent viral DNA in the liver, with or without detection in the plasma. As we tested viral DNA only in plasma samples, these individuals may still be OBI carriers with undetectable HBV DNA and can therefore transmit the disease to potential receivers.

Another limitation of this work is the restricted number of our cohort, as extending this study to more donors will make the results more statistically significant and, therefore compelling.

5. CONCLUSION

Our data showed that there were a substantial number of blood donors with resolved HBV infection but no anti-HBs antibodies that may be at significant risk for reactivation of infection under conditions such as immunosuppressive treatment, stem cell or kidney transplantation, or anticancer chemotherapy [27], [28].

Besides, in order to enhance the safety of our current strategy of testing prior to blood donation, which solely relies on HBsAg negativity, we highly recommend implementing additional screening measures for HBV antibodies, particularly in older donors.

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AUTHOR CONTRIBUTIONS

TS: conception of the experiment, data analysis and manuscript drafting. MM, MY, FM, CH and KB: contributed in clinical data interpretation.SE and SB: helped in HBV DNA quantification. AH carried out manuscript design and writing.

DATA AVAILABILITY STATEMENT

The datasets are available from the corresponding author on reasonable request.

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CONFLICT OF INTEREST DISCLOSURE
The authors declare no competing interests.

ETHICS APPROVAL STATEMENT
This study was conducted in accordance with the Helsinki Declaration and approved by the ethics committee of the University of Nouakchott (ethics clearance letter No002/2020/CE/UNA). Participants signed an informed consent questionnaire at the time of

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