

## Anti-HBc and HBV-DNA among Blood Donors in North Africa; Western Libya

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors MKAS and FFI designed the study, wrote the protocol and the first draft of the manuscript. Authors FFI and EAF managed the literature searches and helped in discussion writing. Authors MKAS and FFI done the analyses of the study with help of statisticians. Authors NMG, KAA and FA done and supervised the laboratory work. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** Post transfusion hepatitis B (PTHB) continues to be an important public health concern with regard to blood transfusion in Libya and in Africa. This concern is related to the screening test which is still used but it is not enough to detect infective cases during window period.

**Objectives:** To determine the presence of total anti-HBc (hepatitis B core antibodies) and HBV-DNA (hepatitis B viral DNA) (in healthy HBsAg (hepatitis B surface antigen) negative blood donors in Tripoli-Libya, North Africa.

**Methods:** A total of 1256 HBsAg negative blood samples, obtained from healthy blood donors who attended Tripoli's central blood bank, were tested for anti-HBc using the VITROS® 3600

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Immunodiagnostic System. The reactive samples were further tested for the presence of HBV-DNA.

**Results:** From the sample of 1256, 123 (10%) were total anti-HBc positive. Of the 123 anti-HBc positive samples, 13 (10.5%) tested positive for HBV-DNA by PCR (Polymerase chain reaction). The frequency of anti-HBc positive cases among the voluntary donors was 59.2%, and among the replacement donors was 40.7% ( $p < 0.0001$ ). The number of anti-HBc positive samples was found to be particularly high in the age group 30-39 years (44.7%) ( $p < 0.0001$ ).

All the positive PCR samples were from the age groups 20-39 and 40-59 with highest prevalence among 20-29 ( $p < 0.0001$ ). Most occupation who had positivity with anti-HBc and HBV-DNA were civil governmental workers specially militants, free workers and was less in students. Most positive cases were from east of Tripoli the capital (Tagora, SoqAljomaha).

**Conclusion:** The frequency of anti-HBc among this sample was 10% which is high compared with the international findings. The current study estimated the expected exclusion rate of anti-HBc positive donated blood, as this would be an important factor to consider before adopting anti-HBc testing in addition to HBsAg testing as a mandatory screening test to further enhance transfusion safety.

*Keywords: Hepatitis B virus; blood donors; HBsAg; anti-HBc; HBV-DNA; PCR; Libya.*

## ABBREVIATIONS

HBV-DNA : hepatitis B viral DNA

PTHB : Post transfusion hepatitis B

HBsAg : hepatitis B surface antigen

Anti-HBc : hepatitis B core antibodies

PCR : Polymerase chain reaction

positive (which means chronic or acute cases of HBV in the window period) would have been found to be carrying HBV-DNA in their blood when tested using PCR, and thus these donors may be a potential source of PTHB [5-8]. In addition, evidence of PTHB from such donors have been reported by a number of studies, which recommend that the implementation of anti-HBc testing, along with HBsAg testing, of blood donors would help to detect additional HBV-infected donors and improve the safety of blood transfusion specially in countries with high prevalence of HBV [9-12].

## 1. INTRODUCTION

Hepatitis B virus infection (HBV) continues to be a major public health problem. More than 240 million people worldwide have chronic hepatitis B infection, leading to the death of more than half a million people each year due to its serious consequences [1]. The prevalence of chronic HBV carriers in Libya is considered to be within the lower limit of the intermediate zone of HBV epidemicity as classified by WHO [2], with a prevalence rate of 2.2% [3].

Considerable efforts have been made by the health authorities in Libya to track and prevent the disease. Perhaps the most important steps have been the adoption of the hepatitis B vaccine for all newborns within the national immunization programme started in the early 1990s, and the screening of blood donors for the presence of HBsAg in the 1980s [4]. However, screening for HBV infection in blood donors in Libya is limited to HBsAg testing, even though several studies have clearly demonstrated that a percentage of donors who are HBsAg negative but anti-HBc

Such screening is important especially when considering the lack of an advanced testing system for HBV, such as nucleic acid testing (NAT). The present study has been conducted to determine the seroprevalence of anti-HBc in blood donors in western Libya (Tripoli area), as well as to estimate the presence of HBV-DNA in blood samples that are positive for anti-HBc but negative for HBsAg, and to estimate the exclusion rate of the anti-HBc positive donated blood. This would be an important factor for the health authorities to consider before adopting anti-HBc testing in addition to HBsAg testing as a mandatory screening test to increase transfusion safety.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

The study was a Cross sectional study.

## 2.2 Area

North West of Libya, Tripoli the capital.

## 2.3 Time

Done in 2014 for one year.

## 2.4 Ethical Consideration

The study protocol was reviewed and approved by the Ethical Committees of National Authority for Scientific Research (NASR) of Libya. All participants endorsed a written informed consent form.

## 2.5 Study Population, Design and Sample Size

A total of 1256 blood samples were obtained from healthy blood donors who attended Tripoli's central blood bank during the year 2014. This blood bank serves neighboring cities as well as Tripoli. The donors were interviewed and medically examined before transfusion, as per the blood bank's standard operating protocol; any donors who were anaemic, or who had low body weight or low blood pressure at the time of donation, were excluded. All the donors were counseled and informed about the study, and consent was obtained from each donor to collect an anonymous sample of serum to be used in this study.

Anonymous questionnaires were completed by each donor, which included personal and demographic data. Donors who were donating blood to their relatives or friends were classified as replacement donors, and donors who were donating blood voluntarily were classified as voluntary donors.

## 2.6 Serological Analysis

All mandatory screening tests for blood-transmitted infections, such as HBsAg, anti-HCV and anti-HIV (anti-HIV-1 and -2), were performed in the central blood bank using the VITROS® 3600 Immunodiagnostic System (France) which is fully automated serologic analyzer. All blood samples which gave a negative result in these tests were simultaneously tested for anti-HBc using the same analyzer in the central blood bank of Tripoli.

## 2.7 Real-time PCR

All anti-HBc-reactive samples were then further tested by PCR for the presence of HBV-DNA; 500 µL of each sample was extracted, amplified and target HBV DNA fragment detected using the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 system (analysis conducted in France by Taqman Roche, Cerba). The test procedure was carried out according to the manufacturer's instructions. The sensitivity of the real-time PCR used is 20 IU/mL; the conversion factor is 1 IU= 5.82 copies [13,14]. The real-time PCR was performed once.

## 2.8 Data Statistical Analysis

Was performed using Statistical Package for Social Science (SPSS) computer software (Version 19, SPSS Inc. USA). The contributing blood donors were divided into age groups. Data was calculated and described by using mean, mode, standard deviation, cross tabulations and graphical presentations. A chi-square test was performed to examine and compare the seroprevalence of anti-HBc between age groups.

## 3. RESULTS

The majority of the donors were males (1248, or 99.4%) and 8 (0.06%) were females. Their age range were from 16 to 93 years old (mean age 34±8.9). Donors occupations were concentrated mainly in civil governmental workers specially militants, free workers and students. The donor population had not previously been screened for anti-HBc.

The donors were from different regions of the Tripoli metropolitan area like Tagora, SoqAljomaha in the east, Alfernag, Almadina Alrithia in the center and Alsrage, Hayalandlas in the west. Of the total 1256, 653 (52%) were voluntary donors who had donated more than once before and have very high risk to transfer hepatitis to others if not diagnosed during the window gap, and 603 (48%) were replacement donors, who were donating only for their relatives or friends for the first time are less dangers because they are not repeaters of blood donation as they may infect their relatives only.

Anti-HBc screening was performed simultaneously with the other mandatory screening tests for blood transmitted infections; 123 samples gave positive results for anti-HBc, giving an overall prevalence 10%.

The frequency of anti-HBc positive cases among the voluntary donors was 59.3% (73 persons), and among the replacement donors it was 40.7% (50 persons) ( $p < 0.0001$ ) (Fig.1). The number of anti-HBc positive samples was found to be particularly high in the age group 30-39 years (44.7%) ( $p < 0.0001$ ) (Fig. 2 & Table 1).

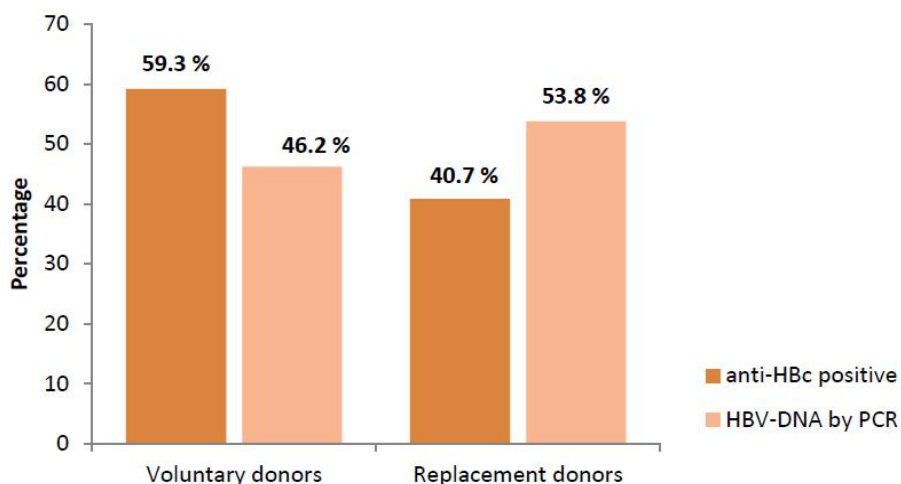


Fig. 1. Frequency of anti-HBc and HBV-DNA in voluntary and replacement donors

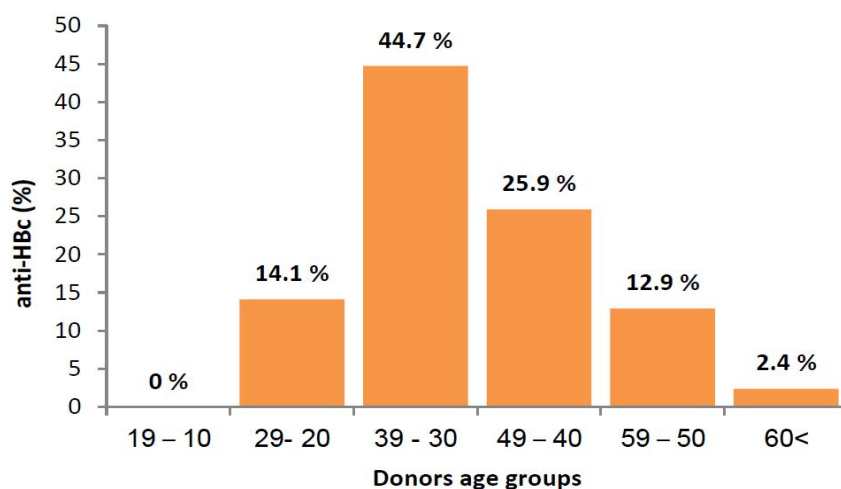


Fig. 2. Percentage of anti-HBc among different age groups

Table 1. Showing age groups screened by total anti-HBc and PCR

Age group	Number of donors screened	Anti-HBc positive cases among whole samples	PCR positive cases (HBV-DNA) among anti-HBc positive cases
10-19	20	0	0
20-29	406	17 (13.8%)	4 (23.5%)
30-39	475	55 (44.7%)	3 (5.5%)
40-49	257	32 (26%)	5 (15.6%)
40-49	80	16 (13%)	1(6.3%)
40-49	18	3 (2.4%)	0
Total	1256	123 (10%)	13 (10.5%)

All anti-HBc positive samples were tested by quantitative real-time PCR. Thirteen samples were found to test positive for HBV-DNA. This figure represents 1% of the whole sample and 10.5% of the anti-HBc positive samples. All the positive PCR samples were from the age groups 20-39 and 40-59 (Fig. 3) with highest prevalence among 20-29 years old ( $p < 0.0001$ ) (Table 1). The frequency of HBV-DNA positive cases among the voluntary donors was 46.2%, and among the replacement donors was 53.8%. This confirms that replacement donors in this sample had high positivity rate of HBV-DNA compared to volunteers ( $p < 0.0001$ ) (Fig. 1).

Most occupations had positivity with anti-HBc and HBV-DNA were civil governmental workers specially militants and free workers but less in students. Most positive cases were from east of Tripoli the capital (Tagora, Soq Aljomaha).

Exclusion rate for anti-HBc positive donated blood was calculated and estimated that approximately one hundred blood units would be excluded from every thousand donated units if anti-HBc testing were adopted. Moreover, the study estimates that ten donated units per thousand may potentially be infected with HBV.

#### 4. DISCUSSION

Currently, HBsAg is the only serological marker used for the screening of blood donors for HBV infection in most blood transfusion centers in Libya. This study investigated the prevalence of anti-HBc and HBV-DNA in healthy blood donors who had tested negative for HBsAg and other mandatory blood-transmitted infection screening in Libyan transfusion centers. The study examined 1256 healthy blood donors who attended the central blood bank in Tripoli and found that the frequency of anti-HBc among this sample was 123 (10%). This percentage was low in comparison with the findings of a previous pilot study (15.6%) that was conducted by the same authors in the same region, though this difference may be due to the difference in study size [15]. However, the percentage was similar to the results of a preliminary study (9.8%) conducted by the authors in the same place earlier in the same year (2014) [16].

A similar prevalence rate (10.96%) of anti-HBc among HBsAg-negative blood donors was

reported in the neighboring country, Egypt [17], although another study in Egypt reported a lower prevalence rate (7.8%) [18]. The reported prevalence of anti-HBc is lower in areas of low endemicity in Europe, where the reported percentages vary between 0.07% in the UK and 1.5% in Germany [19] and are slightly higher (4.85%) in Italy [20].

In contrast, the prevalence has been found to be high in Kuwait (17%) [21], in the Kingdom of Saudi Arabia (15.32%) [22] and Iran (6.5%) [23], and very high (42%) in Sudan. This may be due to the fact that HBV infection is highly endemic in Sudan, as classified by WHO [24].

The prevalence of HBV-DNA in anti-HBc positive donors was 10.5%; this figure was high compared with the previous pilot study (3%) conducted by the authors in the same region [15]. This percentage is similar to that found in Egypt (11.54%) [17]; however, it is higher than that reported (6.25%) in another study in Egypt [18] and higher than the 4.86% reported in Italy [20]. It is broadly similar to the 12.2% found in Iran [23]. These differences could be related to the endemicity of HBV infection among different countries.

The detection of HBV-DNA in anti-HBc positive samples "anti-HBc alone" may be due to chronic unresolved infection with low grade, possibly intermittent virus production and persistent HBV infection, or it may signify the window period in new HBV infection during which anti-HBc is the only detectable marker of recent hepatitis infection. The infection of HBV can be transmitted from HBsAg negative individuals through blood transfusion or organ transplantation [25-27].

As this study estimates that approximately one hundred blood units would be excluded from every thousand donated units if anti-HBc testing were adopted and ten donated units per thousand may potentially be infected with HBV. We recommend that the introduction of anti-HBc screening, at least for first-time donors, would help to detect further infected donors and improve the safety of blood donation. In addition, the introduction of anti-HBc testing would help to identify more chronic HBV carriers; this may allow early access to therapy and thus prevent the serious consequences of HBV infection.

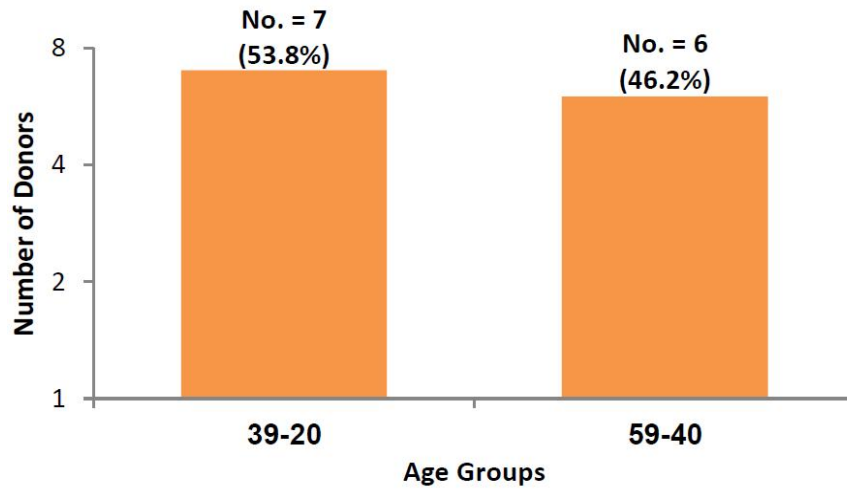


Fig. 3. HBV-DNA positive samples among the age groups

## 5. CONCLUSION

In conclusion, the prevalence rate of anti-HBc in this study (10%) was low in comparison with a previous study in the region. However, the study emphasizes the importance of implementing anti-HBc in addition to HBsAg testing as mandatory screening tests to further increase transfusion safety. The study also recommends that donated blood from donors who are HBsAg negative but anti-HBc positive should be discarded. In addition, the study has estimated the exclusion rate for anti-HBc positive donated blood, which is an important factor for health authorities to consider before adopting anti-HBc testing as an additional mandatory screening test to enhance the safety of transfusions.

## 6. STRENGTHS and LIMITATIONS OF THE STUDY

It is the first Libyan big laboratory based study that used anti-HBc and PCR to detect the positivity of hepatitis B disease among blood donors in Libya. Moreover, it uses enough sample size, thus, the result produced from this study reflect the real situation in the Libyan populations living in the capital Tripoli but cannot be generalized among the whole general blood donors of Libya.

## 7. RECOMMENDATION

To do other studies to measure anti-HBc and PCR in other parts of Libya specially the South

as there is a high level of immigration status in that area.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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