



## Serological Markers of Hepatitis B Virus in Blood donors in Alkhoms, Libya

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العلامات المصلية المضادة للفيروس التهاب الكبد البائي في المتبرعين بالدم في الخمس-ليبيا

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### الملخص

هدفت هذه الدراسة إلى الكشف عن الأجسام المضادة للمستضد اللبي لفيروس التهاب الكبد B (anti-HBc) بين المتبرعين بالدم السلبيين للمستضد السطحي للفيروس (HBsAg) والتحقق من وجود الحمض النووي للفيروس (HBV-DNA) في العينات الإيجابية. شملت الدراسة 102 متبرع جميعهم كانوا من الذكور من مصرف الدم بمستشفى سوق الخميس-الخمس، تراوحت أعمارهم بين 17-56 سنة، وتم فحص العينات باستخدام تقنية (ELFA) للكشف عن anti-HBc، واستخدمت تقنية (RT-PCR) للكشف عن HBV-DNA. أظهرت النتائج أن جميع العينات كانت سالبة لفحص HBsAg، في حين وجدت ثلاث عينات إيجابية لفحص anti-HBc وكانت اثنان من هذه العينات من فصيلة الدم O+ بينما واحدة من الفصيلة A+، لم تسجل فروق معنوية بين النتائج وموقع السكن أو فصيلة الدم ( $P < 0.05$ ). كما أظهر اختبار RT-PCR وجود عينة واحدة موجبة لـ HBV-DNA، مع وجود علاقة ذات دلالة إحصائية بين وجود anti-HBc وكشف الحمض النووي الفيروسي.

**الكلمات الدالة:** التهاب الكبد البائي، المستضد السطحي HBsAg، المستضد اللبي anti-HBc، الحمض النووي الفيروسي HBV-DNA، المتبرعين بالدم، ليبيا.

### Abstract

This study aimed to detect antibodies against the Hepatitis B virus core antigen (anti-HBc) among blood donors who tested negative for the Hepatitis B surface antigen (HBsAg), and to verify the presence of viral DNA (HBV-DNA) in the positive samples. The study included 102 male donors from the blood bank at Souq Al-Khamis Hospital, Khoms, with ages ranging from 17 to 56 years. Samples were screened for anti-HBc using the Enzyme-Linked Fluorescent Assay (ELFA) technique, while the Real-Time Polymerase Chain Reaction (RT-PCR) was employed for the detection of HBV-DNA. The results indicated that all samples were HBsAg-negative; however, three samples tested positive for anti-HBc. Among these, two samples were of the O+ blood group, and one was A+. No statistically significant differences were observed between the results and variables such as residence or blood group ( $P > 0.05$ ). Furthermore, RT-PCR analysis identified one HBV-DNA positive sample,

demonstrating a statistically significant correlation between the presence of anti-HBc and the detection of viral DNA.

**Keywords:** Hepatitis B Virus (HBV), Surface Antigen (HBsAg), Core Antibody (anti-HBc), Viral DNA (HBV-DNA), Blood Donors, Libya .

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## **Introduction**

### **1. Introduction**

Viral hepatitis affects millions of individuals worldwide, posing a significant threat to public health. Approximately 500 million people suffer from chronic Hepatitis B Virus (HBV) infection, with an estimated one million deaths occurring annually due to the virus. Furthermore, it is estimated that HBV infection accounts for 57% of liver cirrhosis cases and 78% of primary liver cancer cases [1, 2].

Despite the availability of a vaccine and blood screening protocols for infection markers, HBV remains a serious health challenge [3]. Its multiple transmission routes, including mother-to-child transmission during childbirth, sexual contact, and direct exposure to blood or blood products [4], exacerbate the severity of the virus. Notably, the HBV vaccine was incorporated into Libya's national immunization program in 1993 [5].

Post-Transfusion Hepatitis B (PTHB) remains one of the most common transmission routes and continues to be reported despite the mandatory screening for Hepatitis B surface Antigen (HBsAg) [6, 7]. This is primarily attributed to Occult Hepatitis B Infection (OBI), which occurs in individuals who carry the viral DNA despite the absence of HBsAg in their blood or hepatic cells [8].

Detecting antibodies against the Hepatitis B core antigen (anti-HBc) during this phase serves as a valuable serological marker for identifying infection [9]. Specifically, the presence of anti-HBc IgM indicates an acute infection, typically becoming undetectable within six months. In contrast, anti-HBc IgG remains detectable for life, indicating either a previous or current HBV infection [10]. Current protocols in Libya do not require anti-HBc screening among blood donors, notwithstanding warnings from numerous studies that recommend its inclusion in routine screening [11].

This study aims to determine the prevalence of anti-HBc among HBsAg-negative blood donors and to evaluate the feasibility of adopting anti-HBc testing as an additional screening measure. Furthermore, the study seeks to detect viral DNA (HBV-DNA) using Real-Time Polymerase Chain Reaction (RT-PCR) in all samples that test positive for anti-HBc and negative for HBsAg.

### **2. Materials and working methods**

In this cross-sectional study, a total of 102 HBsAg-negative male blood donors from the Souq Al-Khamis Hospital Blood Bank in Khoms were screened. The study aimed to detect antibodies against the Hepatitis B core antigen (anti-HBc) and to subsequently identify Hepatitis B virus DNA (HBV-DNA) among anti-HBc positive individuals. Formal ethical approvals and informed consent were obtained from all participants after they completed a structured questionnaire regarding their personal and medical history.

From each donor, 5 mL of venous blood was collected. Following coagulation, serum was separated via centrifugation at 3,000 rpm for 10 minutes and stored at -70°C until further analysis. Routine screenings for HBsAg, anti-HIV, and anti-HCV were performed. Samples that initially tested negative for these markers were further analyzed for anti-HBc using the Enzyme-Linked Fluorescence Assay (ELFA) technique (BioMérieux, France).

Subsequently, anti-HBc positive samples were tested for HBV-DNA using Real-Time Polymerase Chain Reaction (RT-PCR) with the HBV Real-TM QualiQ kit (NLM, Italy), which has a sensitivity of  $10^5$  IU/mL. Statistical analysis was conducted using the Social Sciences Statistical Package (SPSS, version 24.0, SPSS Inc., Chicago, USA). The Chi-square test was employed to compare various variables and to evaluate statistically significant correlations between them.

### 3. Results

This cross-sectional study included 102 male blood donors, with ages ranging from 17 to 56 years (mean age:  $34.77 \pm 8.00$  years). All participants underwent screening for antibodies against the Hepatitis B core antigen (anti-HBc), in addition to the routine Hepatitis B surface antigen (HBsAg) test. None of the donors tested positive for HBsAg. The majority of the donors (68.63%) were aged between 20 and 39 years. Participants were residents of various areas within Khoms, with the highest proportion (27.45%) originating from the Souq Al-Khamis region.

#### 3.1 Descriptive Statistics

The age distribution of donors showed that the majority (68.63%) were within the 20–39-year age group. The normality of the age variable was assessed using the Shapiro–Wilk test, which indicated that age did not follow a normal distribution ( $p < 0.05$ ), as shown in Figure 1.

Regarding blood group distribution, the majority of donors belonged to blood group O+, with 36 donors (35.3%), followed by blood group A+ with 28 donors (27.5%). The remaining blood groups were represented at lower frequencies. Other blood groups showed relatively low distributions, ranging between 5 and 10 donors per group, as illustrated in Figure 2.

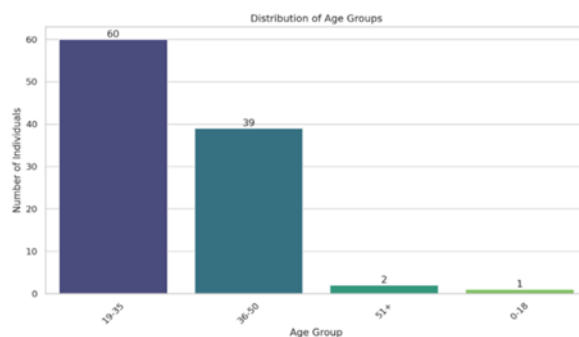


Figure 1. Age distribution of blood donors

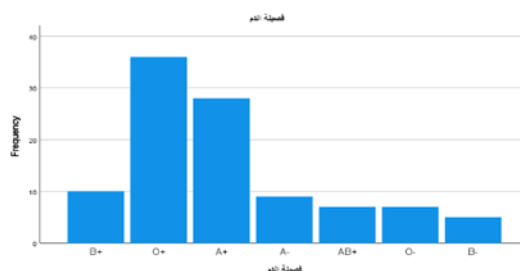


Figure 2. Distribution of blood types among donors

### 3.2 Serological and molecular tests:

Among the 102 donor samples, the results showed that three donors aged between 21 and 60 years tested positive for anti-HBc, with a prevalence of 2.94%. These cases were distributed across different blood groups and age categories, with no statistically significant differences observed, as presented in Tables 1 and 2.

Regarding the place of residence of the positive cases, no statistically significant association was found between anti-HBc positivity and residential location.

Molecular analysis revealed the presence of HBV-DNA in one of the three anti-HBc-positive samples, corresponding to 33.33% among anti-HBc-positive individuals. The donor who tested positive for both anti-HBc and HBV-DNA was 40 years old and belonged to blood group O+, resulting in a positivity rate of 2.78% within this blood group.

The Chi-square test demonstrated a statistically significant association between anti-HBc positivity and HBV-DNA detection ( $P = 0.005$ ), indicating that the presence of anti-HBc is associated with an increased likelihood of detecting occult hepatitis B infection (OBI), thereby emphasizing the importance of its inclusion in blood screening protocols.

**Table 1. Anti-HBc positivity by age group**

Age Group	Total Donors	Anti-HBc Positive	Positivity Rate(%)
>20	1	0	0
21-40	70	1	1.43
41-60	31	2	6.45

$$\text{Positivity rate (\%)} = (\text{Number of anti-HBc-positive donors} / \text{Total number of donors}) \times 100$$

**Table 2. Anti-HBc positivity according to blood type**

Blood Group	Total Donors	Anti-HBc Positive	Positivity Rate(%)
O+	36	2	5.56%
A+	28	1	3.57%
Other Groups (A-, AB+, B+, B-, O-)	38	0	0%

### 4. Discussion

In this study, which is considered the first of its kind in the city of Al-Khums and was conducted to highlight the causes of occult infection with this type of virus and the potential risk arising from limitations in routine screening tests for detecting possible sources of infection among blood donors, the results showed that the prevalence of anti-HBc positivity among 102 HBsAg-negative blood donors was 2.94%. This rate is comparable to findings from local studies conducted in Misrata and Zliten (3.9% and 3%, respectively) [11,12], while it is lower than that reported in another local study in Tripoli, which recorded a higher prevalence of 15.6% [13].

At the Arab regional level, the prevalence was lower than that reported in Saudi Arabia (17.4%), Egypt (10.96%), and Syria (10.32%) [14–16], but it was close to findings from Lebanon (2.2%) [17].

At the global level, the results differed from studies conducted in Iran (8%) and Bangladesh (16.3%) [18,19], while they were consistent with studies from northern India (3.2%) and Pakistan (2.2%) [20,21], and comparable to a study from Nigeria (5%) [22]. This variation in anti-HBc prevalence between countries may be explained by differences in the endemicity levels of hepatitis B virus infection, according to the World Health Organization classification.

The results of this study also showed that among the three anti-HBc-positive samples, one sample was positive for HBV-DNA, representing 33.33%. This finding is comparable to a study from Iran (29.7%) [18], but differs from studies conducted in Saudi Arabia (1.25%) [7] and Nablus (92%) [23].

At the demographic level, the highest rate of anti-HBc positivity was observed in the 41–60-year age group.

## 5. Conclusion

The findings of this study highlight the importance of incorporating anti-hepatitis B core antibody (anti-HBc) testing into routine blood donor screening, due to its significant role in reducing the risk of transfusion-transmitted occult hepatitis B virus infection. Although the implementation of this test may lead to the exclusion of some blood units that appear otherwise healthy, the preventive benefit outweighs this limitation, as it contributes to improving the safety and quality of blood transfusion services.

Furthermore, conducting future large-scale studies using more sensitive techniques for detecting hepatitis B viral DNA (HBV-DNA) is essential to provide a more accurate assessment of the prevalence of occult infection among blood donors in Libya. Expanding epidemiological research to analyze demographic and environmental factors, while increasing sample size to enhance the generalizability of findings, is also recommended.

In addition, raising awareness among blood bank personnel regarding the interpretation of anti-HBc results within the context of complementary testing is crucial to avoid the potential oversight of occult hepatitis B infection (OBI).

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