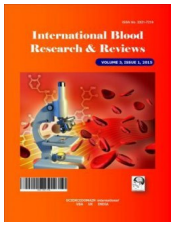

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Occult Hepatitis B Virus Infection among Blood Donors; North Middle Libya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MKAS and FFI designed the study and wrote the protocol along with the first draft of the manuscript. Authors FFI and EAF managed the literature searches and helped in discussion writing. Authors MKAS and FFI have done the analyses of the study with the help of statisticians. Author MAE done the English editing. 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 in regard to blood transfusion in Libya. The inclusion of tests specifically for the hepatitis B surface antigen (HBsAg) began as part of the mandatory screening of blood donors in the early 1980s. This endeavor significantly enhanced blood safety in terms of protecting people against the transmission of an HBV infection. However, several studies have revealed that a percentage of HBsAg donors who tested negative may in fact still be positive for the illness due to a presence of different antigens known as hepatitis B core antibodies (anti-HBc) meaning that it constitutes a possibility of circulating hepatitis B viral DNA (HBV-DNA), thus being a potential source of post transfusion hepatitis B (PTHB).
Objectives: To determine the presence of anti-HBc and HBV-DNA in healthy HBsAg negative blood donors in the middle northern region of Libya (composed of the metropolitan cities of Misrata,

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Khoums, Zlitin, Sirite and surrounding small villages).
Methods: The Misrata central blood bank (which services the middle northern region of Libya) provided A total of 979 HBsAg negative blood samples from healthy blood donors that were tested for anti-HBc using the VITROS® 3600 Immunodiagnostic System. The reactive samples were then further tested for the presence of HBV-DNA.
Results: From the sample of 979, 38 of whom (3.9%) were anti-HBc positive. One sample tested positive for HBV-DNA by PCR (polymers chain reaction) from the anti-HBc positive samples indicating (2.6%) of the subgroup of anti-HBc positive samples and 0.1% from the whole sample population screened.
Conclusion: Despite blood donors being HBsAg-negative in the middle northern region of Libya, 3.9% had anti-HBc, of which 2.6% of the anti-HBc positive donors had detectable HBV DNA. Further studies are needed to determine the actual yield of including an anti-HBc test in routine screening of blood donors.

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 : Polymers Chain Reaction

1. INTRODUCTION

The Hepatitis B virus continues to be a major public health problem in Libya [1]. More than 240 million people worldwide have a chronic hepatitis B infection, resulting in the deaths 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 epidemic as classified by WHO [2].

The prevalence of an HBV infection in Libya was estimated at 2.2% which was done in 2004 among 65,000 blood samples covering all regions of the country (WHO- Libyan CDC) [3,4,5].

In Libya, screening of HBV among blood donors is still done by HBsAg testing [5] which started in the early 1980s [4]. This program began by the Libyan health authorities in an attempt to reduce and prevent the disease by compulsory vaccinations of children and health workers [3,4].

Even though several studies have clearly demonstrated that a percentage of donors are HBsAg negative, some of them have been found to be not only anti-HBc positive but may also be carrying HBV-DNA in their blood when tested using the PCR (polymers chain reaction) technique, and thus these donors can be a potential source of PTHB [6-10].

In Libya, this information is supported by a study done by Shambesh et al. [6] in the Northwestern region, which corresponds to a sample of 1256 of blood donors screened for anti-HBc, 123 (10%) were anti-HBc positive. Of the 123 anti-HBc positive samples, 13 (10.5%) tested positive for HBV-DNA by PCR (polymers chain reaction). Giving an overall prevalence of 1% positivity which would mean that every hundredth blood tranfusion given would be infected indicating a serious source of post transfusion hepatitis B (PTHB) in the population [6].

In addition, evidence of PTHB from such donors has been reported in the northwestern region of Libya and by a number of studies elsewhere, leading to a recommendation in the implementation of anti-HBc testing, along with HBsAg testing, of blood donors in order to help detect additional HBV-infected donors and improve the safety of blood transfusion [6,11-18].

Using anti-HBc screening is especially important 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 the middle northern region of Libya (Misrata and surrounding areas), as well as to estimate the presence of HBV-DNA in blood samples that are positive for anti-HBc but negative for HBsAg, as well as being able to estimate the exclusion rate of anti-HBc positive donated blood.

2. MATERIALS AND METHODS

2.1 Study Design

The study was a Descriptive Cross sectional study.

2.2 Area

The middle northern region of Libya, composed of the metropolitan cities of Misrata, Khoums, Zlitin, Sirite and surrounding small villages.

2.3 Time

Done in 2015 for three months from 1/10/2015 to 31/12/2015.

2.4 Ethical Consideration

The study protocol was approved by the ethics committee of the Scientific Research Authority (SRA) of Libya. Patients who participated in the study all gave written informed consent before being recruited.

2.5 Study Population, Design and Sample Size

A total of 979 blood samples tested were collected from the central bank of Misrata which covers middle northern region of Libya (Misrata and their neighboring cities).

All HBsAg-negative blood donors were included and exposed to the standard operating protocols of the national blood banks of Libya for their interviews, medical examinations and exclusion criteria to identify those who are not fit medically for donations.

Standard questionnaires were used for each donor that included personal, demographic and medical data. Persons donated for their families were classified as family –replacement donors, and those who gave blood voluntarily were classified as voluntary-free donors.

2.6 Serological Analysis

All blood samples were tested for HBsAg. Similtaneously, negative samples were also tested for anti-HBc using the automated serologic analyzer, VITROS® 3600 Immunodiagnostic System (analysis done in France).

2.7 Real-time PCR

The test was done in France after 500 µL of each positive HBc sample was extracted to be amplified, tested, and then scanned to detect DNA fragments 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 [15,16].

2.8 Data Statistical Analysis

All data was stored, analyzed and presented using the Statistical Package of Social Sciences software (Version 19, SPSS Inc. USA). A chi-square test was used to highlight the statistical significance in differences between variables.

3. RESULTS

Among blood samples tested (979), the majority of the donors were males (974, or 99.5%) and only 5 (0.5%) were females. Their ages ranged from 16 to 76 years of age (mean age being 31±7.7). The donors had not been previously tested for anti-HBc.

Samples of blood donations given came from citizens residing in the middle northern region of Libya (consisting of the land beginning from Garaboly, 35 KM east of Tripoli, the capital, to Sirte, another 500 Km east, encompassing Misrata and surrounding towns and villages). Of the total 979, only 94 (9.6%) were voluntary-free donors who had donated more than once before and 885 (90.4%) were family replacement donors.

Anti-HBc screening was done for the blood samples (by default, all were HBSAg negative); 38 samples gave positive results for anti-HBc among the 979, giving an overall prevalence of 3.9%.

HBc positive cases among the voluntary-free donors were 8.1% (three persons), and among the family replacement donors were 91.9% (35 persons) (p<0.0001) (Fig. 1). The majority of anti-HBc positive samples were found mainly in the age group 18-49 years with a concentration in the age group of 20-29 (43.2%). (p<0.0001) (Fig. 2 and Table 1).

Table 1. Showing screened age groups by total amount listed anti-HBc positive

Anti-HBc positive cases among whole samples	Number of donors screened	Age group
10.8	4	10-19
43.2	16	20-29
27.0	10	30-39
18.9	7	40-49
100.0	37	Total

PCR tests were done to all anti-HBc positive donors, only one blood donor was positive giving an overall prevalence of 0.1% of the whole sampled population and listed as 2.6% of the anti-HBc positive samples. The case tested positive for HBV-DNA was a family donor, male and young, being twenty years of age.

Samples tested anti-HBc positive showed relationship with their respective occupations. Prevalence among professions consisted of private businessmen (40.5%), bureaucrats/ civil servants (32.4%), teachers (10.8%), students (10.8%) and others (5.4%) (Table 2). Most positive cases were from outside of metropolitan Misrata (740,75.6%).

The rate of anti-HBc positive exclusion was estimated that approximately 30 blood units would be excluded from every thousand donated units if anti-HBc testing were to be adopted. Moreover, this study estimates that less than ten donated units per thousand may be potentially infected with HBV in the middle northern region of Libya.

Table 2. Showing different occupations among anti-HBc positive cases

%	No. of cases	Occupation
40.5	15	Free workers
32.4	12	Civil workers
10.8	4	Teachers
10.8	4	Students
5.4	2	Others
100	37	Total

4. DISCUSSION

In Libya, HBV Serology among blood donors is only restricted to the presence of HBsAg [5,6]. This study tested 979 healthy blood donors in middle northern region of Libya by detecting another type of HBV antigen known as anti-HBc and found 38 positive samples giving a prevalence of 3.9%. This percentage of 3.9% is low compared to that found in 2010 by investigations performed with the same authors in a pilot study done in the northwestern region of Libya (encompassing Tripoli and its surroundings) [17] also this anti-HBc percentage is low compared to that found by shambesh, etal. 2015 which was discovered at 10% in the northwestern region of Libya [6,18].

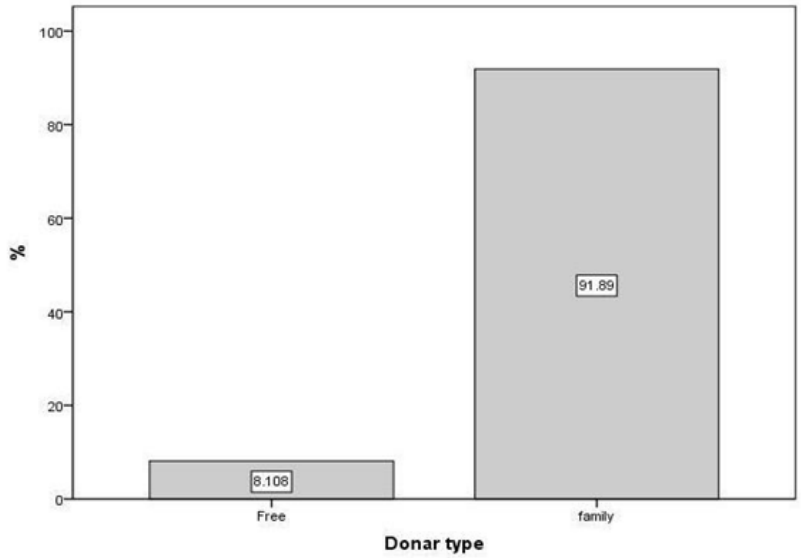

Fig. 1. Frequency of anti-HBc in voluntary-free and family replacement donors

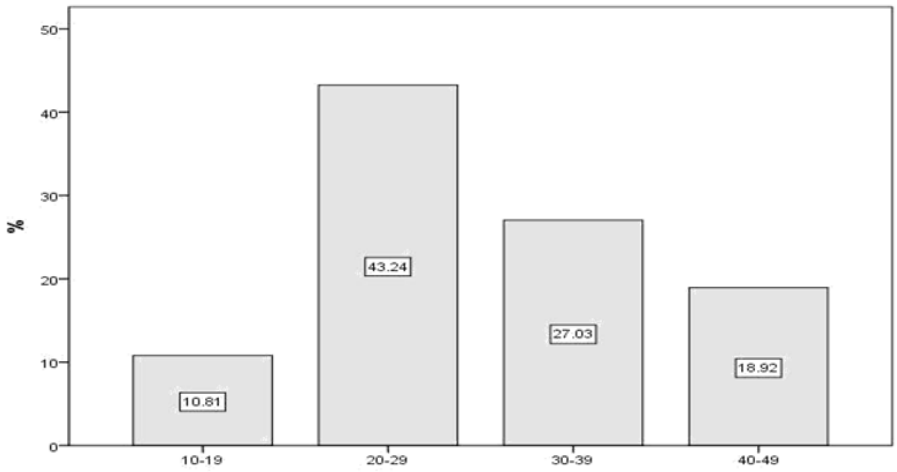

Fig. 2. Percentage of donors tested anti-HBc positive among different age groups

Prevalence of HBc has also been reported reported from countries neighboring Libya; Egypt (7.8% and 10.9% in two different studies) [19,20]. Although the North African countries show high prevalence in an HBc presence, in Europe the prevalence show lower levels endemic to that part of the world; varying between 0.07% in UK, 1.5% in Germany [21] to 4.9% in Italy [22].

In the Middle East, the prevalence of HBc among blood donors has been found to be higher; 17% in Kuwait [23], 15.3% in Saudi Arabia [24]. In countries elsewhere, the prevalence varies between 6.5% in Iran [25] and a very high prevalence (42%) in Sudan [26].

This variations of HBc levels between different countries were related to the different levels of HBV infections being endemic as classified by WHO, Libya was classified in the middle level of the endemic range, Europe was classified with low levels of being endemic while Asian countries and some African countries were classified with high levels [2].

2.6% of HBV-DNA detected among HBc positive donors was low compared with the previous study (10.5%) conducted by the same authors in the Tripoli region [6-18]. The difference in this percentage between the Tripoli and Misrata regions could be related to the higher Hepatitis B levels endemic to Tripoli (1.7% & 2.9% respectively) [5] and also that most of blood donated in Misrata was classified as family types which usually had lower levels of infectivity. This percentage is also lower to that found in Egypt (11.54%) [19] and lower than the 4.86% reported in Italy [22]. It is substantially lower to the 12.2% found in Iran [25].

The importance of this study and other similar studies using anti-HBc detection techniques will help facilitate early diagnoses of patients having HBV infections without showing HBsAg in their blood. This antigen not only signifies the window period in recently infected patients but also identifies those patients with chronic persistent infections. Both of which are attributed with low levels of HBsAg making it difficult to be diagnosed unless using specific tests for anti-HBc or HBV-DNA [27,28].

This study which was done in the middle northern region of Libya estimates that approximately 40 blood units should be excluded from every 1000 donated units (referring to being anti-HBc positive) and one donated unit per 1000 (1/1000) may be potentially infected with HBV-DNA. This estimated rate is lower than the rate calculated in the Libyan northwestern region of 123/1000 anti-HBc and 13/1000 of HBV-DNA potentially infected to the denoted population [6,17,18].

5. CONCLUSION

In conclusion, the prevalence rate of anti-HBc in this study was high (3.9%), but with a low level of HBV-DNA at 0.1%. The exclusion rate is also high, estimated in the vicinity of 40/1000 positive anti-HBc blood donations samples and 1/1000 could potentially be infected with HBV-DNA.

6. STRENGTHS AND LIMITATIONS OF THE STUDY

This is one of the biggest Libyan laboratory based studies that uses anti-HBc and PCR to detect the presence of the hepatitis B disease among blood donors in Libya. Moreover, this analysis uses a large enough sample size, thus, the results produced from this study reflect the real situation of the Libyan population living in the middle northern region of Libya but cannot be generalized among the whole population of blood donors in Libya.

7. RECOMMENDATION

This study emphasizes and recommends the implementation of anti-HBc testing in addition to pre-existing HBsAg inspection as a mandatory screening evaluation 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. Moreover, Studies should be done to measure anti-HBc and HBV-DNA in other parts of Libya with particular interest to the South (such as Sabha) and the North East (including Benghazi) as there are a high levels of immigration status in that area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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