Original Article

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Screening of Occult Hepatitis B Virus Infection among Egyptian Blood Donors.

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Abstract

Background: Although sensitive screening assays for hepatitis B virus surface antigen (HBsAg) are available, special cases of post-transfusion hepatitis B virus infection still occur. The present study was conducted to evaluate the prevalence of anti-hepatitis B core (anti-HBc) positivity and the presence of HBV-DNA in serum samples of healthy blood donors negative for both HBsAg and anti-HCV antibodies in Benha, Egypt.

Materials and methods: The study included a screening of 450 selected blood donors. The distribution of blood donors was 288 males (64%) and 162 females (36%). The recruited blood donors who met the criteria for blood donation were routinely screened for HBsAg, HIV I/2-Ab, and syphilis-antibodies. The blood units for donations were further analyzed for the presence of HBc-IgM and HBV-DNA levels by PCR method.

Results: Testing of the accepted units for the donation was about 12 (2.7%) HBc-IgM positive, and 9 (2%) HBV-DNA positive units. The standard screening of blood unit failed to recognize early acute or window HBV infection where HBsAg is missing.

Conclusions*:* Our investigations proposed that sensitive methods for the detection of HBV by PCR might be recommended in the screening of donated blood. Furthermore, anti-HBc antibodies should be tested regularly on all blood donation units.

Introduction

In recent years, there has been increased community concern about the precautions of blood transfusion. HIV-1, HIV-2, hepatitis B and hepatitis C stay infectious causes by transfusion and are related to essential clinical illness [1]. The possibility of transfusion-transmitted hepatitis B virus (HBV) infection has been done by screening all blood donations for hepatitis B surface antigen (HBsAg) since 1970 [2]. Antibodies to hepatitis B core (HBc) antigen is the sign of acute, chronic, or resolved HBV infection and persist noticeable for lifespan [3]. These can be available without both HBsAg and anti-HBs antibodies, during the recuperating time frame following acute hepatitis B before the presence of antibodies of HBs, or in patients who developed the disease. Antibodies to HBc are recognized in any individual who has been contaminated with HBV [4].

The incubation period of HBV infection range from 45 to 160 days. After acute disease, the main virologic marker is the envelope protein and HBsAg. As a rule, serum HBsAg can be gone before rises in serum transaminases as clinical side effects as well as stayed visible during the acute icteric period [5]. The HBsAg gets invisible 1 to 2 months following the beginning of jaundice. After HBsAg disappearance, the antibodies to HBsAg disclose in serum and stay perceptible [6].

There could be a lag period (window) between the disappearance of HBsAg and the presence of antibodies of HBsAg, during which time patients with HBV disease may not be distinguished by routine serologic testing. The hepatitis B core antigen (HBcAg) itself is sequestered inside the HBsAg coat and isn't typically perceptible in the serum of HBV- patients [7]. Antibodies to HBc (IgM), however, might be perceptible and can be used to recognize acute HBV disease during the window period [8].

It has been shown that HBsAg negative people and those positives for antibodies to HBc keep on restoring HBV. These discoveries recommended that convalescence from acute hepatitis B infection disease may not bring about complete infection, however rather the humoral immune response keeps the infection at a low level [9].

It is known, in any case, that some blood subsidiaries, negative for HBsAg yet positive for antibodies against hepatitis B core antigen (anti-HBcAg), can transmit the disease, both after transfusion and after transplantation of organs [10]. HBcAg antibodies with no other HBV serological marker is as often as possible found in a various population. Various circumstances may represent this outcome false-positive antibodies of HBcAg result; low degrees of HBV replication inside the hepatocyte, without evidence of HBsAg; the window period of acute HBV disease; the loss of antibodies to HBsAg with time; or the nearness of an immunization get away from vaccine escape mutant, not recognized by the majority of available HBsAg detection tests [11].

As per the sensitivity of polymerase chain reaction (PCR) technique has been improved, people assigning HBV DNA as the main marker of contamination have been discovered [12]. Occult hepatitis B disease is characterized as the presence of HBV DNA in the blood of hepatitis B surface antigen (HBsAg), with or without antibodies to hepatitis B core antigen (anti-HBc) or hepatitis B surface antigen (anti-HBs). Occult hepatitis B was found in 13-71%

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of liver tissue and in 5-55% of sera from patients with chronic liver disease, who tested negative for HBsAg and antibodies to HCV [13]. To put it briefly, for the current study, we make an effort to test the eligible blood units for unraveling the acute HBV infection by using HBV-DNA level by PCR along with serological markers of blood donations.

Materials and methods

The study was carried out at the Regional Blood Transfusion Center (RBTC), Benha, Egypt, and movable blood collection vehicles through September 2019. Donors were questioned and physically inspected before blood transfusion. Persons who met donor collection conditions were designated for blood donation. Serum samples were stored at -20ºC pending procedure. It was conducted on 450 newly diagnosed healthy blood donors' volunteers.

Routine serological assay

All blood specimens were tested on a sequential basis for routine serological tests. The routine serological tests according to the predefined protocol of blood banking safety requirements by the Ministry of Health and Population (MOHP) issued 1994, comprised HBsAg, anti-HCV, and anti-HIV-l/2 as well. Detection of HBsAg was done using a commercially available ELISA kit (Axiom). HBsAg-positive samples were retested using a commercially available enzyme immunoassay kit (MonoLISA, Bio-RAD, France).

Detection of HCV-Ab was done using the commercially available 4th generation ORTHO HCV ELISA test system. HCV-Ab-reactive samples were confirmed by antibodies against HCV ELISA kit, 4'h generation commercial kits (HCV; Abbott murex). Detection of anti-HIV-l/2 antibodies was done using a commercially available Bio-Rad GENSCREEN HIV-1/2 kit.

Qualitative and Quantitative Analysis of HBV

Eligible units for transfusion were tested for anti-HBc antibody IgM (HBc IgM) using commercially available Clinotech Diagnostics HBcAb IgM EIA 3rd generation for the detection of IgM antibody. Detection of HBcAb IgM using DiaSorin kit was done to confirm positive samples detected by the Clinotech Diagnostics HBc IgM EIA kit. Detection of HBV-DNA was done using PCR. Briefly, l0 ul extracted DNA was added to a reaction mixture composed of 8 ul 10 mM dNTP, l0 ul 10x *Taq* DNA polymerase buffer, 100 mmol of outer sense ('5-GTCTGTGCCTTCTCATCTGCC-3') and antisense C5-AGAATAGCTTGCCTGAGTGC-3) primers, and 1 ul (5U) *Taq* DNA polymerase. The reaction mixture was adjusted to 100 ul using H_2O. The amplification protocol was 4 minutes at 95ºC, followed by 30 cycles composed of 95ºC for one minute (denaturation), 55ºC for one minute (annealing), and 72ºC for one minute (extension).

Statistical analysis

Data were analyzed using the SPSS computer program version 22.0. Quantitative data were expressed as means ± standard deviation. Qualitative data were expressed as numbers and percentages. Chi-Square test and Fisher's Exact Test were used when appropriate for comparison between qualitative variables.

Results

The studied sample included 450 selected blood donors with 288 males and 162 females. Participation of volunteers were in urban residence 300 volunteers with percentage (66.7%) and 150 volunteers from the countryside (33.3%) as shown in **(Table 1)**.

Donors of ages between 19 and 30 years constituted the largest proportion of males (64%) and 36% of females were tested for the routine serological viral markers. All donors exhibited normal blood pressure, pulse rate, and were voluntary non-compensated blood donors, and were competent by a feedback form official by MOHP. All volunteers were tested for HbsAg, HCV, HIV, and syphilis as prerequisites before blood donation, all tests came in the negative results **(Table 2)**.

Out of all volunteers, and by furthered investigations, 12 samples (2.7%) were reported as reactive for HBcIgM along 4 samples were positive for HBeAg. According to an analysis of HBeAb and PCR came with positivity for 3(0.7%) and 9 (2.0%) of volunteers respectively **(Table 3)**.

HBV-DNA by PCR was detected in 12/438 (33.3%) donations. By comparison of results of HBeAg and HBeAb applied to HBc IgM reactive, were positive in 4 samples (33.3%) and negative in 8 samples (66.7%) in HBeAg. As per HBeAb, 3 (25%) samples came positive and 9 samples (75%) from 12 samples were positive in HB_CIgM **(Table 4)**.

All volunteers who have been diagnosed with HBcIgM positive were classified according to Sociodemographic criteria **(Table 5)**.

Discussion

Transfusion of infected blood by viral hepatitis is a major health problem. Screening blood donation for viral markers is an important source of information about the epidemiology of these infections [14]. The study was aimed to measure the age, sex, and blood group-specific prevalence of HBV in apparently asymptomatic healthy individuals donating blood. Consistent with routine practice, blood donor volunteers were questioned and medically inspected before contributing. Persons with high-risk behavior together with intravenous drug addicts, past of immoral sexual relations, homosexuals, or those with any medical problem particularly jaundice, bleeding disorders requiring constituent transfusion, pregnancy or recent delivery less than 12 weeks were disallowed [15]. Persons were also methodically asked about HBV vaccination. Recognized donors were regularly separated serologically for the presence of HBsAg, HCV-Ab, HIV-l/2-Ab, and Syphilis antibodies. Normally, negative donations for the above revealed serological markers are considered eligible for transfusion. It was found that among the accepted volunteers for donation Males outnumbered females in the present study group.

Table 1. Socio-demographic variables of the studied blood donors.

Variable	No.	Percentage (%)
Gender:		
Male	288	64
Female	162	36
Residence:		
Urban	300	66.7
Rural	150	33.3
Total	450	100

Table 2. Distribution of the blood donors according to serological tests for some diseases (N = 450)

Serological test	Reactive No. (%)	Negative No. (%)	Total No. (%)
HBsAg	0 (0)	450 (100)	450 (100)
HCVAb	0 (0)	450 (100)	450 (100)
HIVAb1+2	0 (0)	450 (100)	450 (100)
Syphilis	0 (0)	450 (100)	450 (100)

Table 3. Distribution of blood donation according to the results of HBsAg negative (N = 450)

HBV viral markers	Reactive No. (%)	Negative No. (%)	Total No. (%)
HBcIgM	12 (2.7)	438 (97.3)	450 (100)
HBeAg	4 (0.9)	446 (99.1)	450 (100)
HBeAb	3 (0.7)	447 (99.3)	450 (100)
PCR	9 (2)	441 (98)	450 (100)

Table 4. Results of HBe Ag, HBe Ab, and PCR testing applied to HBC IgM reactive blood donors (N = 12)

HBV viral markers	Reactive No. (%)	Negative No. (%)	Total No. (%)
HBe Ag	4 (33.3)	8 (66.7)	12 (100)
HBe Ab	3 (25)	9 (75)	12 (100)
PCR	9 (75)	3 (25)	12 (100)

Table 5. Distribution of HBC IgM reactive blood donors according to their Sociodemographic criteria (N = 12).

Sociodemographic criteria	Results
Age	
Mean± S.D.	30.8 ± 6.2
Median(Range)	30.50 (23–40)
Gender	
Males N (%)	8 (66.7)
Females N (%)	4 (33.3)
Education	
Undergraduate N (%)	1 (8.3)
Bachelor N (%)	5 (41.7)
Intermediate diploma N (%)	6 (50)
Residence	
Urban N (%)	6 (50)
Rural N (%)	6 (50)

Out of 450 blood donors, 288 (64%) were male and 162 (36%) were female. This is in contrast to the study conducted by Said *et al*. in Egypt, where males (83.6%) outnumbered females (16.3%). This variance can be accounted for by the fact that in Egypt, females are less educated, socially less exposed, and are less enquired for blood donation. In consequence, there is a superior sum of male blood donors in study [16]. Our outcomes revealed that from the total 450 blood donor samples only 2.7 % anti-HBc IgM was noticed. Acute hepatitis B is categorized by the concurrent existence of HBsAg and anti-HBc IgM. Through recovery, anti-HBc IgM is present during the window period within HBsAg disappearance and anti-HBs antibody [17]. These results are in agreement with the results of a recent study by Zhou *et al* [18]. The study showed 0.9 % and 0.7 % seroprevalence of HBeAg and HBeAb respectively among blood donors with HBc IgM positive test. The rate is comparable to that reported in Mohammed *et al*. that the highest prevalence of 37.5% (9/24) for HBeAg was found among blood donors within the age group 17- 26 years; blood donors within age group 27-36years had the lowest prevalence of 20.0% (2/10) while no HBeAg was detected among blood donors within the age group 47-56 years and ≥ 57 years and the prevalence of 15.6% for Hepatitis B e antibody HBeAb [19]. The factors showing these differences may be due to education, socioeconomic level, and awareness of the population. The occurrence may also vary according to the method designed for testing and the size of the sample taken in the study. Moreover, in the current study 9 (2%) cases were positive for HBV-DNA. These cases were chosen negative for all serological markers used in this study. According to, the distribution of HBc IgM reactive patients, the study showed that the mean age of total 12 positive HBcIgM was 30.8 ± 6.2 and the frequency of HBcIgM was found to be 66.7% in males and 33.3% in female's blood donors. A higher incidence in males than females could be for the reason that males more repeatedly volunteer for blood contribution. An additional reason could be that females do not achieve the minimum standards essential for blood contribution, this is in agreement with the study done by Japhet *et al*., (2011). The education level for those whom HBcIgM were positive appeared in intermediate education levels with equality in residence by 50% in both sexes [20].

Conclusion

To sum up, screening for occult hepatitis B infection must be a routine practice in blood donors. Anti-HBc antibody tests should be included in routine tests done on blood donor volunteers to reduce HBV post-transfusion transmission.

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