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Anti-HB Core Screening Significance among Healthy Blood Donors in Fayoum, Egypt

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Abstract

Background: Occult Hepatitis B is the existence of HBV-DNA in the serum of HBsAg negative cases with or without the presence of antibodies to HBV. Although (HBV)transmission viahepatitisB surface antigen (HBsAg) negative blood donors has beenreported, still HBsAg is the only obligatory HBV screening test of blood donors in Fayoum.

Aim: Expanding the donor screening procedure to include anti-HBc to reduce the risk of transfusion transmitted HBV infection.

Methods: A total of 400 HBsAg negative blood donors were included in the study from blood bank, Fayoum University Hospital, Egypt. All donors were tested for anti-HBc, and HBsAg-negative, anti-HBc -positive sera were further tested quantitatively for antibodiesto hepatitis B surface antigen (anti-HBs). 'anti-HBc alone' sera were examined quantitatively for HBV by real time polymerase chain reaction (qPCR). Moreover, liver function tests and some oxidative stress parameters were determined in the sera of Anti HBc –ve group and Anti HBc +ve groups.

Results: Among 400 HBsAg -negative donors, 69 (17.2%) were anti-HBc -positive, of which 12 donors (17%) were HBsAb negative, 20 donors (29%) were HBsAb low positive and 37 donors (54%) were HBsAb high positive. The 12 'anti-HBc alone' donors displayed 2 cases (16.6%=0.5% of total) with HBV positive DNA. On comparison of the mean liver function profiles of Anti HBc –ve group and Anti HBc +ve group, no statistically significant difference was observed.

Conclusion: These data suggest including anti-HBc as an additional screening test for blood donors in Fayoum.

Keywords: Anti-HBc; Blood donors; Occult HBV infection

Introduction

Transfusion plays an important role in the supportive care of medical and surgical patients. Transfusion-transmitted infectious diseases remain a major topic of interest for those involved in blood safety. Globally, the most notable transfusion-related risks are human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) due to their high prevalence rates [1].

Hepatitis B virus (HBV) remains a major public health problem [2]. It is estimated that approximately 400 million people worldwide are chronically infected with HBV, where Egypt is considered as an area of intermediate endemicity [3]. The risk of transmitting hepatitis through transfusions of blood and blood products has been known since 1950 [4,5]. In 1965, Blumberg reported on the discovery of the hepatitis B surface antigen (HBsAg) [6]. In 1970, Purcell identified the hepatitis B virus (HBV) [7].

The presence of antibodies against the hepatitis B virus core (anti-HBc), in the absence of both the hepatitis B surface antigen (HBsAg) and the hepatitis B surface antibody (anti-HBs), is evidence of a chronic HBV infection, which remains detectable for life [8,9]. Usually, an HBV infection is diagnosed with the detection of HBsAg and anti-HBc in the serum or plasma of an individual [10,11].

Occult HBV infection initially described in the late 1970 by [12]. Occult HBV infection is characterized by the presence of HBV DNA in blood or tissues with undetectable HBsAg and with or without the presence of anti-HBs [13,14]. The highestof occultvirus inwas reported among patients with hepatocellular carcinoma and similar to the scenario for classicinfection where genotype D is the most prevalent genotype [15].

HBV is one of the most common causes for chronic liver disease (CLD) in the developing countries. The virus is known to be highly infective and is associated with long-term morbidity and mortality due to complications like cirrhosis, portal hypertension, and hepatocellular carcinoma (HCC). Moreover, between 10% and 30% of patients with chronic hepatitis B experience flares that resemble acute hepatitis B. Flares are characterized by a short-lived rise in levels of a liver enzyme (alanine aminotransferase), which is caused by the destruction of infected hepatocytes by the immune system. Since flares reflect an immune response to the virus, they frequently coincide with the development of antibodies against the antigen (HBcAg). Between 8%

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and 15% of patients with chronic hepatitis develop antibodies (Anti-HBV) against HBcAg [16].

Oxidative stress condition arises during imbalance between oxidants and antioxidants of diverse origin damaging both structure and function of tissues [17]. In infectious diseases, inflammatory cells have been shown to become activated and secrete reactive oxygen and nitrogen species. There have been various studies indicating that oxidative stress is increased in hepatitis B and hepatitis C infections and in liver disease [18,19]. Oxidative stress increases and antioxidant values decreases as the disease becomes chronic in patients with hepatitis B infection [20].

Most blood banks does not screen routinely for anti-HBc in the blood donors. Anti-HBc positivity indicates past HBV infection. Replacement of this blood in an immune-compromised individual can result in reactivation of the virus. So, the present study aim to determine the prevalence of the Hepatitis B Core antibody among voluntary blood donors in Fayoum, Egypt and highlight the significance of screening anti-HBc to reduce the risk of transfusion transmitted HBV infection in Blood banks.

Methods and Materials

Study population

A cohort cross-sectional prospective study included 400 healthy blood donors assigned at the blood transfusion center of Fayoum University. The blood donors were either voluntary or replacement donors. The selected donors were healthy according to their clinical histories, and physical examinations, and they fulfilled the suitability criteria for donation. Five milliliters of peripheral blood were drawn. The specimens were kept at room temperature for half an hour, and then centrifuged at 489 X g for 15 min. Serum was separated and then stored at -40^{0}C until tested. Written informed consents were obtained from all enrolled donors at the time of sampling. The age of the studied donors ranged from 17 to 60 years. A clinical epidemiological interview was performed with all the patients.

Serological assays

All sera were tested for HBsAg, by enzyme-linked immunosorbent assay (ELISA) according to the routine practice in Fayoum university hospital blood bank. Additionally, antibodies to hepatitis B core antigen (anti-HBc) were tested in all samples. HBsAg negative, anti-HBc positive sera were further tested quantitatively for antibodies to hepatitis B surface antigen (anti-HBs) and sera were considered negative, low-positive or high positive when anti-HBs titers were <10 IU L^{-1}, between 10 and 100 IU L^{-1} or >100 IU L^{-1} , respectively. 'anti-HBc alone' sera were considered for HBV quantitative real time polymerase chain reaction (qPCR).

HBV DNA detection

Viral nucleic acids were extracted using (RTP DNA/RNA Virus Mini kit (STRATEC Molecular GmbH, D-13125 Berlin) according to the manufacturer's instructions. HBV DNA quantitative real-time PCR was carried out targeting the surface gene region [21] and using LC-Fast Start DNA Master HybProbes kit on the LightCycler instrument (Roche Diagnostics). The final 20 µL volume reaction mix contained 0.2 µm of each primer (forward, 5_-CTTCATCCTgCT gCTATgCCT-3_; reverse, 5_-AAA gCC Cag gATgATggg AT-3_), 0.2 µm of Taq Manprobe (5_-FAM-ATg TTg CCC gTTTgT CCT CTA ATT CCA.-BBQ 3_; TIB MOLBIOL, Berlin, Germany), 5 mm of $MgCl_2$ and 2 µL of purified DNA. Thermal cycling profile was initiated at 95^{0}C for 10 min followed by 50 amplification cycles at 95^0 C for 10 s and 60^{0}C for 24 s.

Serum biomarkers for liver function tests

The appropriate kits (Bio-Diagnostic, Dokki, Giza, Egypt) were used for the determination of total protein by colorimetric method according to method described by Tietz [22]. Albumin is determined using colorimetric end point method according to modified bromocresol green binding assay (BCG). Serum aminotransferase enzyme activities (AST&ALT) were measured according to Reitman and Frankel [23-25].

Statistical analysis

Data were analysed using SPSS computer software (SPSS 16, 2008; SPSS Inc., Chicago, IL, USA). The chi-squared test was used on the observed versus expected frequencies in the distribution of the hepatitis markers, corresponding to different characteristics. P-values less than 0.05 were considered statistically significant.

Results

Over a period of two months (July 2014 to Sept 2014), a total of 500 blood units were collected at the blood transfusion center of Fayoum University, amongst them, a total of 400 first time voluntary blood donors were potentially eligible for participation in the study (Figure 1). Among the 400 HBsAg -negative donors, 69 (17.2%) were anti-HBc-positive [sample-to cutoff (S/CO) ratio: 6.76Å [3.35], hence were eligible for further investigation revealing 37 anti-HBs high positive and 20 anti-HBs low-positive donors (Figure 1). The remaining 12 donors were 'anti-HBc alone', two of which (~17%) were HBV DNA-positive with viral loads of 2.5 E+4 copies mL^{-1} and 6.4 E+4copies mL^{-1}.

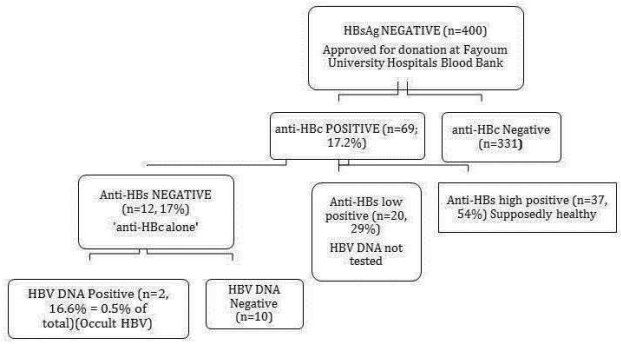

Figure 1: Schematic representation of the study population.

The mean age of the study subjects was 29.3 ± 7.9 y with age ranged from 17-60 y. Maximum donations were observed in the age group of y, with male donation per cent and female donations per cent, respectively (Table 1). No statistically significant difference was observed amongst male and females with respect to age, anti- HBc and anti-HBs status (Table 2).

Oxidative stress markers, malondialdehyde (MDA), reduced glutathione (GSH) levels as well as catalase (CAT) activity in Anti HBc –ve group and Anti HBc +ve group are illustrated in Figure 2. Level of MDA was significantly increased in Anti HBc +ve group as compared to Anti HBc –ve group (Figure 2A). On the other hand, GSH level and CAT activity decreased significantly in Anti HBc +ve group as compared to Anti HBc –ve group (Figures 2B and 2C).

Age (year)	Male (n=376)	94%	Female (n=24)	6%	Total (n=400)	100%
<=20	31	8.2%	5	20.8%	36	9%
21-30	171	45.5%	11	45.9%	182	45.5%
31-40	140	37.2%	6	25%	146	36.5%
41-50	24	6.4%	2	8.3%	26	6.5%
>=50	10	2.7%	0	0%	10	2.5%
Anti HBc -ve (Group I)	311	82.7%	20	83.3%	331	82.8%
Anti HBc +ve (Group II)	65	17.3%	4	16.7%	69	17.2%
Group I + Group II	376	100%	24	100%	400	100%

Table 1: Serological profile according to age and sex distribution of all blood donors.

Discussion

It is generally accepted that the diagnosis of infection by HBV is based on the presence of the HBsAg in the bloodstream [26]. However, the risk of HBV infection through blood transfusion does not totally eliminated by screening the blood bank donors for HBsAg [27], since the absence of this marker in the serum does not exclude the presence of HBV DNA [28]. In our study, 400 HBsAg-negative healthy donors were approved for blood donation at the Fayoum university hospitals blood bank. Dramatically, 69 of which were anti-HBc -positive, namely17.2% of the accepted blood units were potentially infectious especially donors showing 'anti-HBc alone' serological profile.

Moreover, occult HBV infection was proved in two 'anti-HBc alone' donors (16.6%) with low viral load. However, the actual prevalence of occult HBV infection with 'anti-HBc alone' serological profile might be higher as previously discussed [29]. Evidently, by missing anti-HBc testing in the current serological screening policy in Fayoum university hospitals blood bank, there is a risk to accept blood units with occult hepatitis B infection. This estimate may even increase considering the anti-HBc-positive, anti-HBs low-positive subjects who cannot be excluded from potential occult HBV infection.

Previous studies estimated that the total costs of anti-HBc tests to prevent one case of transfusion-based HBV transmission is much lower than the average costs required for HBV diagnosis, treatment and follow-up for one patient. Thus employing anti-HBc test as a preventive measure was shown to be cost-effective, though a proportion of anti-HBc-positive donations might be non-infectious due to past HBV infection [30] or minimal cross-reactivity [29,31]. The reduction of blood unit's pool is insignificant compared to the risk of HBV transmission and its serious consequences particularly among immune compromised recipients. Moreover, as previously discussed, the practicality of anti-HBc testing would be further enhanced if quantitative anti-HBs testing is additionally considered only foranti-HBc-positive donors, and those with high anti-HBs titers exceeding 100 IU L−1 [32] are generally non-infectious anti-HBc-positive blood units, thus could be retained to save lost blood units.

Implementation of HBV nucleic acid testing in Fayoum university hospitals blood bank will be very costly and unaffordable by a poor community like Fayoum even by implementing mini pools strategy (MP-NAT); particularly that potentially infectious anti-HBc positive donations with extremely low DNA levels might not be detected even by sensitive ID-NAT [33]. Therefore, utilizing NAT for HBV detection is neither feasible nor affordable at Fayoum university hospitals blood bank.

Parameter	**Anti HBc –ve n=331**	**Anti HBc +ve n=69**
AST (5-45 IU/l)	35.4 + 4.3	36.3 + 3.7
ALT (5-55 IU/l)	42.3 + 5.1	45.1 + 4.3
ALP (30-125 IU/l)	92.1 + 9.4	87.3 + 8.1
Total bilirubin (0.2-1.3 mg/dl)	0.65 + 0.10	0.78 + 0.21
Total protein (6-8 g %)	5.6 + 0.40	6.1 + 0.32
Data are mean + SEM; ALT; Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; *P<0.05		

Table 2: The mean liver function profile between Anti HBc –ve group and Anti HBc +ve group.

The present study highlights the need of using several markers rather than a single marker during screening of HBV infection. Reactive oxygen intermediates (ROS) are involved in many of the complex interactions between the invading microorganisms and its host [34]. Oxidative stress in the pathogenesis of hepatitis virus might be caused by a combination of chronic inflammation, liver damage and proteins encoded by the virus [35]. Oxidative stress develops when a disturbance in balance between ROS produced in excess and factors preventing their harmful effects occur. A number of studies have linked hepatitis virus proteins or viral hepatitis to the development of oxidative stress [36,37]. Two prominent markers to monitor oxidative stress during viral infection are Malondialdehyde (MDA) and cellular Glutathione (GSH). ROS generated as a result of HBV infection significantly increased lipid peroxidation as reflected by the level of MDA. In accord with the results of Mahdy et al. [38], Çıragil1 et al. [39] and Mehde et al. [40] the intensity of oxidative stress was measured as enhancement in the levels of lipid peroxidation end product, malondialdehyde (MDA) as a result of HBV infection. Increase in serum MDA levels in hepatitis B infected patients may be valuable in monitoring viral hepatitis cases.

Mehde et al. [40] and Li et al. [41] our results support the notion that depletion of blood GSH in HBV infected patients is one of the major factors that permit lipid peroxidation and subsequent tissue

damage. The primary cause accounting for the decreased blood GSH level in patients with liver diseases is a decreased production and decreased inflow from the liver [42]. Moreover, insufficiency in nonenzymatic antioxidant GSH following viral infection in the present study could be the consequence of HBV replication [41].

Levels of antioxidant enzymes, such as SOD-1 and CAT, are closely linked with cellular responses to various forms of oxidative stress. Osman et al. [43] reported that an increase in oxidative stress markers and a decrease in antioxidant enzyme activities were observed in the serum of patients with viral hepatitis. The present study extended the previously reported finding that CAT activity significantly decreased in the serum of HBV infected patients [39,44]. In addition in accord with our results Escobar et al. [45] and Sanzgiri et al. [46] have reported that the enhanced free radical concentration resulting from the oxidative stress conditions can cause loss of enzymatic activity.

In conclusion, our study stressed the immediate need for implementing anti-HBc testing at Fayoum university hospitals blood bank, besides the routine screening for HBsAg, to avoid the devastating undesirable effect of transfusion transmitted HBV.

Compliance with Ethical Standards

There were no external funding sources for this study and there is no conflict of interest with any organization regarding the material discussed in the manuscript.

All procedures performed in our study were in accordance with the ethical standards of Egyptian Network Research Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from each subject before enrolment in the study and the Fayoum University Research Ethics Committee, which is a member of Egyptian Network Research Ethics Committee (ENREC), was informed of this study.

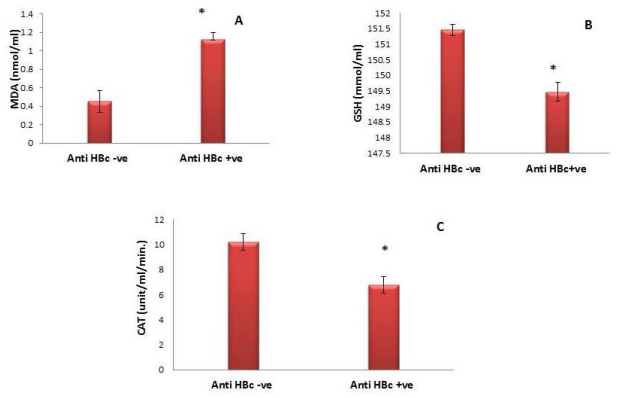

Figure 2: Levels of malondialdehyde (MDA) (A), glutathione reduced (GSH) (B) and catalase (CAT) (C) in anti HBc-ve and anti HBc +ve groups. Values are given as mean ± SEM in each group. *: Values are significantly different (P<0.05)

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