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ABSTRACT We tested the frequency of occult hepatitis B infection (OBI) among Egyptian healthcare workers (HCWs). We tested 132 HCWs for hepatitis B virus (HBV) DNA by nested polymerase chain reaction (PCR), and hepatitis C virus antibody (anti-HCV) by ELISA. HCV RNA was measured by nested PCR in anti-HCV-positive HCWs. HBV-DNA-positive HCWs were subjected to HBV genotyping. We included 132 HCWs who were negative for hepatitis B

surface antigen and positive for hepatitis B core antibody (anti-HBc). OBI was detected in 7 male HCWs, and HBV E genotype was detected in 3, HBV D in 2 and HBV D and E in 2. Two OBI-positive HCWs had a history of neonatal hepatitis B vaccination. Anti-HCV seropositivity was detected in 17 HCWs who were positive for anti-HBc; 15 of whom were positive for HCV RNA by nested PCR. HCV infection was confirmed by anti-HCV and HCV RNA in 1 of 7 HCWs with OBI. In conclusion, Egyptian HCWs have a significant rate of OBI and HBV E genotype is prevalent.

Hépatite B occulte chez des agents de soins de santé égyptiens

RÉSUMÉ Nous avons étudié la fréquence de l'hépatite B occulte chez des agents de soins de santé égyptiens. Nous avons examiné 132 agents de santé à la recherche d'ADN du virus de l'hépatite B (VHB) au moyen de l'amplification en chaîne par polymérase (PCR) nichée et de l'anticorps du virus de l'hépatite C (anti-VHC) par la méthode ELISA. L'ARN du VHC a été mesurée par PCR nichée chez des agents de santé positifs pour l'anticorps anti-VHC. Les agents de santé positifs pour l'ADN du VHB ont été soumis au génotypage du VHB. Nous avons inclus 132 agents de soins qui étaient négatifs pour l'antigène de surface de l'hépatite B et positifs pour l'anticorps antinucléaire de l'hépatite B (Anti-HBc). Une hépatite B occulte a été détectée chez 7 agents de santé de sexe masculin, et le VHB de génotype E a été détecté chez trois agents, le VHB de génotype D chez 2 agents, et le VHB de génotype D et E chez 2 agents. Deux des agents positifs pour l'hépatite B occulte avaient été vaccinés durant la période néonatale. Une séropositivité anti-VHC a été détectée chez 17 agents de santé qui étaient positifs pour l'anticorps anti-HBc, dont 15 étaient positifs pour l'ARN du VHC par PCR nichée. L'infection à VHC a été confirmée par l'anti-VHC et par l'ARN du VHC chez 1 agent sur 7 atteints d'hépatite B occulte. En conclusion, les agents de santé égyptiens ont un taux d'hépatite B occulte significatif et le génotype E du VHB est prévalent.

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Received: 20/07/16; accepted: 25/10/16

Introduction

Egyptian healthcare workers (HCWs) are at increased risk of infection with bloodborne pathogens. Countrywide baseline assessment in 2001 revealed that government and private hospitals had no occupational safety programmes. A 2002 survey of Egyptian HCWs revealed unsafe practices in the use and disposal of sharps and reported that HCWs were frequently exposed to needle stick injuries. This problem is aggravated by low hepatitis B vaccination coverage, which was only 14% among Egyptian HCWs (1).

Occult hepatitis B infection (OBI) is marked by the presence of hepatitis B virus (HBV) nucleic acid in serum and/or liver tissue, with hepatitis B surface antigen (HBsAg) seronegativity. The worldwide prevalence of OBI ranges from 1 to 95% (2), depending on HBV endemicity, the method of detection of HBV DNA, and the studied risk group. The frequency of OBI among HCWs ranges from 0 to 11% (3–6).

Egypt had a high prevalence of hepatitis C virus (HCV) and intermediate endemicity of HBV infection. The prevalence of HBsAg and HCV antibody (anti-HCV) among Egyptian HCWs is 1.5% and 8–16.6%, respectively (7, 8). High frequencies of OBI have been detected among different high-risk populations in Egypt, including blood donors, and patients with chronic hepatitis C, haemodialysis, polytransfusion and haematological and solid malignancies (9). HBV genotypes B–D have been detected among Egyptian OBI patients (9,10). In this study we aimed to clarify the overall prevalence of OBI among Egyptian HCWs.

Methods

Study population

This was a descriptive cross-sectional study conducted between June 2014 and April 2015 among workers of governmental (n = 69) and nongovernmental (n = 63) hospitals in Tanta City, Egypt. These included tertiary (n = 25), secondary (n = 92) and primary care (n = 15) hospitals. All employed staff were invited to participate in the study during an 8-week period. Medical and nursing students on clinical placements were not considered as hospital staff and hence were ineligible for recruitment.

Study recruitment

HCWs were recruited on the basis of accepting study enrolment without any random selection. All HCWs completed the study questionnaire and provided a blood sample for HBsAg testing. Among 556 HCWs negative for HBsAg, 132 (23.7%) were positive for hepatitis B core antibody (anti-HBc) and included in the study. A single study-trained researcher collected data from all study participants. Data on personal demographics, and risk factors for bloodborne infections were collected.

Blood sampling

For each study participant, 5 ml of venous blood was collected. Each sample was centrifuged within 6 hours of collection, divided into 2 serum aliquots and stored at –21 °C for subsequent testing.

Antiviral serology

HBsAg and anti-HBc were tested by third-generation ELISA. Anti-HBc-positive samples were retested and only samples that were positive on both occasions were considered positive. HBsAg-negative and anti-HBc-positive HCWs (n = 132) were further tested for anti-HCV by third-generation ELISA.

Detection of HBV DNA by polymerase chain reaction (PCR)

HBsAg-negative and anti-HBc positive samples (n = 132) were tested for HBV DNA using nested PCR (core fragment), as described previously (11). DNA was extracted from patients' sera using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). HBV DNA was amplified using two different primer pairs (first set: forward 5′-GTCTGCGGCGTTTTATC-3′; and reverse 5′ACAGTGGGGGAAAGC-3′; second set: forward 5′-TGCCCGTTTGTCCTCTA-3′ and reverse 5′-AGAAACGGRCTGAGGC-3′). Samples from all positive cases were retested to confirm positive results.

HBV genotyping

Seven serum samples that were positive for HBV DNA by nested PCR underwent HBV genotyping. The PCR was conducted as described previously (12) using the primer sequences shown in Table 1 .

HCV RNA isolation and nested PCR

Anti-HCV-positive samples (n = 17) were tested for HCV RNA by nested PCR. Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen). The primer sequences were as follows: forward 5′-CGCGCGACTAGGAAGACTTC-3′ and reverse 5′-ACCCTCGTTTCCGTACAGAG-3′.

Ethics

All procedures followed the regulations of Al-Azhar University Ethics Committee. Informed consent was given by all HCWs participating in the study. Participants were informed of the potential risks and benefits of the study before enrolment. Participants' data were anonymized to optimize confidentiality and privacy, with laboratory data collected and stored only by study number in secure systems. The key record identifying the participant was kept confidential and not made available, except to the principal investigator responsible for dissemination of results to individual participants. Following testing, the principal investigator provided participants with test results on an individual basis.

Statistical analysis

SPSS version 17 was used for statistical analysis. Differences in frequency between groups were compared with the $\chi 2$ test or Fisher's exact test. $P \leq 0.05$ was considered significant.

Results

Characteristics of HCWs

We enrolled 132 HCWs who were HBsAg negative and anti-HBc positive from governmental (n = 69) and nongovernmental (n = 63) hospitals in the Nile Delta. There were 67 (50.8%) women and 65 (49.2%) men, with a mean (standard deviation) age of 37.4 (11.9) years (range 16–64 years). Fifty-nine (44.7%) HCWs provided direct care to patients: 55 (41.7%) nurses, 3 (2.2%) laboratory technicians and 1 (0.8%) physician. Seventy-three (55.3%) HCWs were non-healthcare providers (HCPs): 35 (26.5%) ward workers, 17 (12.9%) administrative officers, 11 (8.3%) security personnel, 9 (6.8%) drivers and 1 (0.8%) cook. The mean duration of healthcare work was 12.63 (7.93) years. Fifteen (11.4%), 8 (6.1%), 2 (1.5%) and 1 (0.8%) of the HCWs had a history of hepatitis B vaccination, prior surgery, type 2 diabetes mellitus and parenteral antischistosomal therapy (PAT), respectively. None of the HCWs had a history of blood transfusion or haemodialysis. Seventeen (12.88%) HCWs were positive for anti-HCV and 115 (87.12%) were negative. Fifteen (88.23%) of these 17 were also positive for HCV RNA by nested PCR.

OBI among HCWs

OBI was detected in 7 (5.3%) of 132 anti-HBc-positive HCWs. All were male with a mean age of 35.85 (13.64) years (range 19–58 years); 4 worked in governmental hospitals and 3 in nongovernmental hospitals; and 3 were nurses and 4 were non-HCPs. The mean duration of healthcare work was 13.8 (7.69) years (range 8–27 years).

None of the HCWs with OBI had a history of surgery, PAT, type 2 diabetes (Table 2), blood transfusion or haemodialysis. Male sex was associated with OBI in our HCWs (P 0.05) (Table 2).

Three of the 7 HCWs with OBI had HBV genotype E, and 2 each had HBV genotype D or genotypes D and E. HBV genotype E was detected in 1 HCW from a governmental hospital and 2 from nongovernmental hospitals. All OBI HCWs with HBV genotype E alone were non-HCPs. HBV genotype D was detected in 2 nurses working at governmental hospitals.

Two of the 7 HCWs with OBI had a history of neonatal hepatitis B vaccination, and both were negative for HCV infection. One of them had HBV genotype E and the other genotype D.

HCV infection was confirmed by both anti-HCV and HCVRNA in 1 of 7 HCWs with OBI.

Discussion

We found that 5.3% of HCWs with anti-HBc seropositivity had OBI. Male sex was associated with risk of OBI in HCWs, yet old age and HCV infection were not. OBI in HCWs was related to HBV genotypes D, E or co-infection with both genotypes. Two HCWs with OBI had prior history of infantile HBV vaccination.

OBI is reported in both upper and lower Egypt. Egyptian patients with chronic hepatitis C have a high prevalence of OBI where the presence of OBI is associated with more progressive liver disease. Reactivation of OBI after chemotherapy has also been reported in Egyptian patients. Anti-HBc has ben identified as a good marker for OBI among different high-risk groups in Egypt (9).

HCWs with OBI may pass undetected and be unaware of their status, placing their patients at risk of infection. There are scarce data on the prevalence of OBI in HCWs worldwide. In the majority of reported studies, the molecular tests were carried out on anti-HBc-positive samples (13). The main finding of our study was that 5.3% of Egyptian HCWs positive for anti-HBc had OBI. This rate is comparable to that reported from India (5%) (14), Poland (4%) (15) and South Africa (6.6%) (16) but markedly lower than the 11% reported by Chiarakul et al. from Thailand (3). The higher OBI rate in the latter study may be attributed to the small sample size. The prevalence of OBI among Egyptian blood donors positive for anti-HBc (6.25–17.2%) is higher than that reported among HCWs in our study (17–19).

The male predominance of HBV infection in HCWs has been reported previously (20). All HCWs with OBI in our study were male, and male sex was identified as a risk factor for OBI. The different course of chronic HBV infection between men and women has long been observed (21). The male to female ratio increased from 1.2 in asymptomatic carriers to 6.3 in chronic liver disease and 9.8 in hepatocellular carcinoma (HCC). Chiaramonte et al. (22) have noted a greater ability in women to produce hepatitis B surface antibody (anti-HBs) in adulthood. It is postulated that male patients with chronic HBV infection might have more frequent HBV DNA integration and subsequent development of HCC (21). Although the ultimate mechanism regulating this difference is not well known, the greater persistence of chronic HBV infection, the low level of anti-HBs production, and greater nuclear integration of HBV DNA in men may explain the male predominance of OBI in our HCWs.

Few studies have examined the frequency of HBV genotypes among Egyptians with OBI. In the current study, we detected the presence of genotype E and coexistence of genotypes D and E among HCWs with OBI. HBV genotype E is considered to exist mainly in East, Central and West Africa. The fact that many students from these countries are studying at Al-Azhar University suggests transmission of HBV genotype E from these students to Egyptian HCWs. The detection of HBV genotype E among Egyptian HCWs with OBI and its absence among other Egyptian risk groups may support hospital-acquired HBV infection among HCWs in our study.

As HBV and HCV share many risk factors and transmission routes, the detection of HCV infection among anti-HBc-positive HCWs with or without OBI is not surprising. El-Sherif et al. (23) showed that, among HBsAg-negative patients, the number with PAT is significantly higher in anti-HBc-positive, HCV-positive patients, compared with anti-HBc-negative, HCV-positive patients. They concluded that PAT transmits both HCV and HBV among many Egyptians.

OBI detection in HCWs may have an impact in several clinical contexts. The possible transmission of the infection from HCWs to patients is an important issue. Compared to overt HBV infection, the routes of transmission of OBI have been studied less. OBI transmission has been reported after blood transfusion, liver transfusion (24) and intrauterine infection in pregnant women with OBI (25). Moreover OBI is transmitted by close contact and manifests clinically (26), which suggests the healthcare setting as a risk environment for OBI horizontal transmission. The contribution of OBI to liver disease progression (26), development of HCC (26), and the risk of HBV reactivation (27) is also important, and suggests screening and treatment of HCWs with OBI. Another relevant factor is the poor response of HCWs with OBI to hepatitis B vaccination. The absence of an immune response after hepatitis B vaccination has been reported among 5–20% of HCWs (28–30). This was attributed to host immune dysfunction (31,32) and viral factors. Indeed, OBI has been documented in HCWs who did not respond to hepatitis B vaccination. This suggests that HCWs with inadequate anti-HBs levels after hepatitis B vaccination should be tested for the presence of OBI by sensitive molecular tests (5).

For prevention of HBV transmission from HCWs to patients, the US Centers for Disease Control and Prevention recommend that HCWs who perform exposure-prone procedures, and who do not have serological evidence of immunity to HBV from vaccination or previous infection, should know their HBsAg status and, if that is positive, should also know their hepatitis B e antigen status. The detection of OBI in HCWs infers that HBsAg may not be an effective indicator of HBV status and suggests nucleic acid testing.

Hepatitis B vaccination is the most effective method for preventing HBV infection. Yet, the protective anti-HBs level decreases over time after neonatal vaccination (33). Although it is generally believed that neonatal hepatitis B vaccination is protective for 15–20 years, and booster vaccinations not required, OBI has been detected in vaccinated people with a mean age of 19–21 years (34). We detected OBI in 2 of 15 HCWs with a history of neonatal hepatitis B vaccination. We recommend that HCWs with such a history should be tested for anti-HBs levels and receive booster vaccination. HCWs with OBI had prolonged duration of healthcare work [13.8 (7.69) years] compared with those without OBI [12.58 (7.97) years], which supports the waning of protective anti-HBs levels over time.

In conclusion, there was a high prevalence of OBI among Egyptian HCWs who were positive for anti-HBc. HCWs with OBI were mainly male and HBV genotype E was the most prevalent. OBI and HCV co-infection were detected and OBI was detected among HCWs with neonatal hepatitis B vaccination. Anti-HBc and/or nucleic acid testing of Egyptian HCWs is recommended to detect and prevent OBI in the healthcare setting. Routine prior hepatitis B vaccination of HCWs and strict infection control measures are mandatory to protect against OBI.

Funding: None.

Competing interests: None declared.

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