Hepatitis B virus genotype E infection among Egyptian health care workers

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ABSTRACT

Background and Objectives: Health Care Workers (HCWs) are at a high risk of needle stick injuries and HBV infection in Egypt; this problem is further aggravated by low Hepatitis B (HB) vaccination coverage. Limited data are available on the prevalence of HBV infection in Egyptian HCWs. In this study, we aimed to assess the HBV infection rate and genotypes among Egyptian HCWs. Methods: Five hundred and sixty-four (564) HCWs were included. Of them, 258 (45.74%) were health care providers and 306 (54.25%) were non-health care providers. All HCWs completed both the study questionnaires and provided a blood sample for HBV testing. Indeed, all HCWs were tested for Hepatitis B surface antigen (HBsAg) and antibody to Hepatitis B core antigen (anti-HBc), by enzyme-linked immunosorbent assay. HBVDNA was checked for HCWs who tested positive for HBsAg and/or anti-HBc, by nested Polymerase Chain Reaction (PCR). HBVDNA positive HCWs were further subjected to HBV genotyping. Results: The mean age of included HCWs was 33.0 ± 9.8 years, of whom 319 (56.56%) were males. The mean duration of health care work was 9.3 ± 6.7 years. The frequency of HBsAg and anti-HBc were 1.4%, and 24.5%, respectively. Old age and prolonged duration of health care work were significantly associated with anti-HBc seropositivity. Among 140 HCWs positive for HBsAg and/or anti-HBc, 14 (10 %) had positive HBVDNA by PCR. HBV/E (n = 7), HBV/D (n = 3) and co-infection with E and D (n = 4) genotypes were detected. **Conclusion**: Egyptian HCWs have a significantly high rate of HBV exposure. The detection of HBV/E genotype among Egyptian HCWs suggests prevalent transmission of HBV/E among Egyptian populations.

Key words: hepatitis B virus, health care workers, anti-HBc, HBsAg, genotype E

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Access this article online

Website:

www.intern-med.com

DOI:

10.1515/jtim-2017-0012

Quick Response Code:



INTRODUCTION

The incidence of HBV infection in Egypt has markedly decreased in the general population after the introduction of the universal infantile Hepatitis B (HB) vaccination in 1992. [1,2] The frequency of HBsAg and anti-HBc positive among Egyptian general populations, with age range of 15-59 years, is 1.5% and 15.7% respectively. [3] HBV genotype D (HBV/D) was reported to be the most prevalent

among Egyptians. Although HBV genotype E (HBV/E) is geographically localized in East, Central and West Africa, it was reported to cause breakthrough infection after HB vaccination in Egyptian populations.^[4]

The risk of HBV infection among HCWs after exposure to Hepatitis B surface antigen (HBsAg) positive blood ranges from 6% to 30%, depending upon the presence or absence of Hepatitis B early antigen (HBeAg). [5,6] Prior to the HB vaccination

era, the frequency of HBsAg and anti-HBs among Egyptian HCWs was 3.2% and 28% respectively. [7] More recently, the frequency of HBsAg was reported to be 1.5% in Egyptian HCWs. [8]

Ideally, HCWs should be vaccinated before entering the professional training programs. [9] Vaccination coverage of Egyptian HCWs is very low. [10] There is no clear policy that requires mandatory vaccination for HCWs in Egypt, as the price of the vaccine is high and the Ministry of Health and Populations cannot afford to vaccinate all staff. [10]

The hepatitis B virus status is not routinely checked among Egyptian HCWs, and limited data are available about the HBV infection rate and genotypes. Here, our objective was to assess the HBV infection rate and genotypes among Egyptian HCWs.

METHODS

Study population

A cross-sectional study was conducted between June 2014 and April 2015 among the workers of governmental (n = 461) and non-governmental (n = 103) hospitals in Tanta City, Egypt. These included both tertiary (n = 257), secondary (n = 191) and primary care hospitals (n = 116). All currently employed staff were invited to the study during an eight-week period. All those reporting for duty during the study period were eligible for enrollment. Medical and nursing students on clinical placements were not considered as hospital staff, and hence, were ineligible for recruitment.

Study recruitment

The recruitment of HCWs was based on the study enrollment acceptance, without any random selection. Overall, 564 HCWs completed both the study questionnaire and provided a blood sample for testing. A single study-trained researcher collected data from all the study participants. Data on personal demographics and risk factors of HBV infection were collected.

Blood sampling

For each study participant, 5 mL of venous blood was collected. Each sample was centrifuged within six hours of the collection into two serum aliquots for storage at -21°C and further testing took place later.

Anti-viral serology

All Health Care Workers were tested for the presence of HBsAg and anti-HBc, by third generation enzyme-linked-immunosorbent assay (ELISA). Anti-HBc positive samples were re-tested and only samples testing positive on the two occasions were considered positive.

Detection of Hepatitis B virus DNA by nested PCR

HBsAg and/or anti-HBc positive samples (n = 140), tested for HBVDNA using nested PCR techniques, as described previously.[11] Briefly, DNA was extracted from the patients' sera using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). HBV DNA was amplified using two different primer pairs (First set: F 5'- GTCTGCGGCGTTTTATC- 3'; and R 5'-ACAGTGGGGGAAAGC- 3'; second set: F 5' - TGCCCGTTTGTCCTCTA- 3' and R 5' -AGAAACGGRCTGAGGC-3'). Samples from all positive cases were retested to confirm the positive results. The first round of PCR was performed with an outer primer set for 35 cycles (94 °C for 40 s, 55 °C for 40 s, and 72 °C for 40 s). The second round was performed with an inner primer set for 25 cycles (94 °C for 40 s, 57 °C for 40 s, and 72 °C for 40 s), followed by the extension reaction. Nested PCR products were subjected to electrophoresis on a 3% agarose gel stained with ethidium bromide, and DNA was observed under ultraviolet light. The detection limit of our nested PCR was 10 copies/mL.

Hepatitis B virus genotyping

A total of 14 HBVDNA-positive serum samples by nested PCR underwent HBV genotyping. The genotyping procedure and sequences of PCR primers were conducted as described before.^[12,13]

All procedures performed in the study were approved by Al-Zagazig University Ethics Committee, and in concordance with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Statistical analysis

SPSS version 17 was used for analysis. Differences in frequency between groups were compared with the chi-square test or the Fisher exact test. A *P* value < 0.05 was considered significant.

RESULTS

Demographic data

Five hundred and sixty-four (564) HCWs were enrolled, relating to both governmental (n = 461) and non-governmental hospitals (n = 103) in Tanta City (Table 1). While 258 (45.74%) of the included workers provided direct health care to patients, 306 (54.25%) were non-health care providers (Table 1). Among the included HCWs, 319 (56.56%) were males and 245 (43.44%) were females, with a mean age of 33.0 \pm 9.8 years (range 16–64). The mean duration of health care work was 9.3 \pm 6.7 years (range 1–30). 9 (1.6%), 13 (2.3%), 15 (2.7%) and 17 (3%) HCWs

had a history of HCV infection, parenteral antischistosomal therapy (PAT), history of type II diabetes mellitus and prior surgery, respectively. Forty-five (8%) HCWs had a history of infantile HB vaccination. None of the included HCWs had a history of adult HB vaccination, blood transfusion or hemodialysis (Table 1).

HBsAg serology

The frequency of HBsAg among the included HCWs was 1.4% (8/564) (Table 2). Of them 62.5% (5/8) worked at governmental hospitals and 37.5% (3/8) worked at nongovernmental hospitals. Of the eight HBsAg positive subjects, two worked at security, two worked as ambulance drivers, one worked at administration department and three

were nurses (Table 2). The frequency of anti-HBc positivity among HBsAg positive HCWs was 75% (6/8), all of them have positive viremia. Thus, two (25%) of the eight subjects with positive for HBsAg were negative for anti-HBc.

Anti-HBc serology

The frequency of anti-HBc among the included HCWs was 24.5% (138/564). Of those, 51.8% (73/138) worked in governmental hospitals, while 47.5% (66/138) worked in non-governmental hospitals. Anti-HBc positive rate in health care providers and none health care providers were 23.64% (61/258) and 25.16% (78/306), respectively. One (33.3%), three (18.75%), and 57 (23.84%) of the included physicians, laboratory technicians, and nurses were sero-

	n (%)		
Total health care workers included	564 (100%)		
Governmental hospital workers	461(81.73%)		
Tertiary care workers	257 (55.74%)		
Secondary care workers	88 (19.08%)		
Primary care workers	116 (28.85%)		
Non-Governmental hospital workers	103(18.27%)		
Tertiary care workers	0		
Secondary care workers	103 (100%)		
Primary care workers	0		
Health care providers	258 (45.74%)		
Physician	3 (1.16%)		
_aboratory technician	16 (6.2%)		
Nurse	239 (92.63%)		
Non-health care providers	306 (54.25%)		
Nord workers	155 (50.65%)		
Officer	68 (22.22%)		
Security	40 (13.07%)		
Driver	34 (11.11%)		
Cooks	9 (2.94%)		
Age (Mean ± SD)	$33.0 \pm 9.8 \text{ years } (16 - 64)$		
emale	245 (43.44%)		
Male	319 (56.56%)		
Duration of health care work (Mean \pm SD)	9.3 ± 6.7 years (1 - 30)		
listory of adult HB vaccination	0		
listory of HB vaccination during infancy	45 (7.97%)		
listory of HBV infection	4 (0.7%)		
distory of HCV infection	9 (1.6%)		
Prior Surgery	17 (3%)		
History of PAT	13 (2.3%)		
listory of DM	15 (2.7%)		
listory of blood transfusion	0		
listory of hemodialysis	0		
lBsAg	8 (1.4%)		
Anti-HBc	138 (24.5%)		
HBVDNA	14/140 (10%)		
HBV genotype E	7/14		
HBV genotype D	3/14		
Co-infection with D and E genotypes	4/14		

SD: standard deviation; HB: hepatitis B; HBV: hepatitis B virus; HCV: hepatitis C virus; PAT: Parenteral antischistosomal therapy; DM: diabetes mellitus.

Table 2: The distribution of HBV markers among different health care categories

	HBsAg Positive	Anti-HBc	HBVDNA
	n (%)	n (%) n (%)	n (%)
Governmental hospital workers ($n = 461$)	5(1.08)	72(15.6)	8(1.73)
Tertiary care workers $(n = 257)$	4(1.55)	29(11.28)	5(1.94)
2^{nd} care workers $(n = 88)$	0	28(31.81)	0
Primary care workers $(n = 116)$	1(0.8)	15(13.27)	3(2.65)
Non–Governmental hospital workers ($n = 103$)	3(2.91)	66(64.07)	6(5.82)
Tertiary care workers	0	0	0
2^{nd} care workers ($n = 103$)	3(2.91)	66(64.07)	6(5.82)
Primary care workers	0	0	0
Health care providers ($n = 258$)	3(1.16)	61(23.64)	6(2.32)
Physicians $(n = 3)$	0	1 (33.3)	0
Laboratory technicians $(n = 16)$	0	3(18.75)	0
Nurses $(n = 239)$	3(1.25)	57(23.84)	6(2.5)
Non-health care providers ($n = 306$)	5(1.63)	77(25.16)	8(2.61)
Word workers $(n = 155)$	0	34(21.93)	1(0.64)
Officers $(n = 68)$	1(1.47)	17(25)	1(1.47)
Security workers $(n = 40)$	2(5)	13(32.5)	2(5)
Drivers $(n = 34)$	2(5.88)	11(32.35)	3(8.82)
Cooks $(n = 9)$	0	2(22.22)	1(11.11)

HCWs: health care worker; HBsAg: hepatitis B surface antigen: Anti-HBc: antibody to hepatitis B core antigen; HBV: hepatitis B virus

positive for anti-HBc respectively. The distribution of anti-HBc sero-positivity among ward workers, officers, security workers, drivers, and cooks were 21.9% (34/155), 25% (17/68), 32.5% (13/40), 32.35% (11/34), and 22.2% (2/9), respectively (Table 2). Older HCWs and those with a longer duration of health care work had significantly higher rates of anti-HBc sero-positivity (Table 3).

HB viremia among HB infected HCWs

Among 140 HCWs positive for HBsAg and/or anti-HBc, HBVDNA was detected in 14 (10%) by nested PCR. 8 (57.14%) HCWs were working in governmental hospitals while 6 (42.85 %) were in non-governmental hospitals (Table 2). 6 (2.51%) nurses and 8 (2.61%) non-health care providers were positive for HBVDNA (Table 2). While the frequency of positive viremia among HBsAg positive HCWs was 87.5% (7/8), it was 8.7% (12/138) among anti-HBc positive. Among the two patients with HBsAg positive but anti-HBc negative, HBVDNA tested positive.

HBV genotypes

Among 14 HBVDNA positive HCWs, HBV/E (n = 7), HBV/D (n = 3) and co-infection with E and D (n = 4) genotypes were detected. Genotype E was detected in governmental and non-governmental HCWs. The mean age of HCWs with HBV/D, HBV/E, and co-infected HBV/D and E genotypes were 28 ± 7.93 , 34 ± 8.5 and 48.5 ± 8.26 years, respectively. In addition, the mean duration of health care work for those with HBV/E, HBV/D and co-infection with E and D were 6 ± 4.35 , 8.7 ± 5.99 and 18.5 ± 7.85 years respectively.

HBV infection among HCWs with infantile HB vaccination

Forty-five (7.8%) HCWs were born after 1992, and thus received infantile HB vaccination. Their mean age was 19.35 ± 1.53 years; there were 21 (46.66%) males and 24 (53.33%) females. Although none was positive for HBsAg, 14 (31.11 %) were anti-HBc-positive and of them, 14.3% (2/14) were HBVDNA positive. Among them, both genotypes D (n = 1) and E (n = 1) were detected.

DISCUSSION

We found that 1.4% and 24.5% of Egyptian HCWs were positive for HBsAg and anti-HBc respectively. Overall, 1.5% and 15.7% of the Egyptian general populations, age 15-59 years, were carriers for HBsAg and anti-HBc, according to the Egypt Health Issues Survey 2015(EHIS 2015). Worldwide, the HBsAg and anti-HBc frequencies in HCWs range from 0.1-8.1% and from 6.2-73.4% respectively. Our findings indicate that exposure to HBV infection in the health care setting is significantly high, but exposure was not followed by chronic infection, mostly due to the adulthood infection.

The prevalence of HBsAg (1.4%) in our HCWs mirrors that of the Egyptian general population, and is significantly decreasing. The declining rate of HBsAg among Egyptian children and general populations was previously reported.^[1,2,15] A study conducted in Egypt between 1986 and 1987 found an HBsAg sero-positivity rate of 3.2% among 765 HCWs.^[7] More recent data published in 2012

Table 3: Risk of anti-HBc positivity among health care workers

		Anti-HBc		t / χ2	Р
		Negative	Positive		
Age		31.61 ± 8.56	37.49 ± 11.78	6.35	< 0.001
Sex					
	Females	176(41.3%)	69(50%)	3.2	0.07
	Males	250(58.7%)	69(50%)		
Duration of	HCW	8.19 ± 5.84	12.52 ± 7.78	6.45	< 0.001
DM		13(3.1%)	2(1.4%)	1.03	0.30
PAT		12(2.8%)	1(0.7%)	2.02	0.15
Health care	provider	62(23.3%)	204(76.7%)	0.366	0.545
Non-health	care provider	76 (25.5%)	222(74.5%)		

Anti-HBc: antibody to hepatitis B core antigen; HCWs: health care workers; DM: diabetes mellitus; PAT: parenteral antischistosomal therapy

showed a significant decrease of HBsAg (1.5%) among Egyptian HCWs in the Nile delta.^[8]

The anti-HBc rate (24.5%) in our HCWs is significantly higher than that reported in Egyptian blood donors negative for HBsAg (7.8-14.2%),^[16-19] and general populations (15.7%).^[3] We could not detect any significant difference of anti-HBc rate between health care providers and non-health care providers. Moreover, a significant increase of anti-HBc frequency among older HCWs, and those with prolonged health care work duration, was detected. Taken together, this supported the suggestion that higher exposure to HBV infection may have occurred in the health care settings.

HBsAg is a product of HBV replication, ccc-HBV-DNA transcription and viral mRNAs translation whereas anti-HBc is an expression of the antiviral immune response against the HBV core antigen. Virtually, all HBsAgpositive are expected to be IgG anti-HBc-positive. Yet, the asymmetry of the two markers is reported among HBV/E genotype. IgG-anti-HBc was detected in 60.7% people among HBsAg positive of Nigerian blood donors, [20] an area with prevalent HBV/E genotype. Two HBsAg positive and anti-HBc negative HCWs were detected in our study. Both were infected by HBV/E genotype and were negative for antibody to Hepatitis C virus and antibody to Human Immune Deficiency virus. Although it is an unusual serologic pattern, the association of HBsAg positive and anti-HBc negative pattern with HBV/E genotype is quite interesting and needs further evaluation for its clinical significance.

In the current study, we detected the presence of HBV/E and co-infection with E and D genotypes, among Egyptians HCWs. Aboushady *et al.* 2011 first reported the breakthrough infection with HBV/E genotype among Egyptian populations with previous HB vaccination.^[4]

According to their results, 54.5% (n=12/22) with HBV/E infection were negative for HBsAg. [4] 72.7% (n=8/11) of our HCWs infected with HBV/E were negative for HBsAg. It was speculated that the presence of HBV/E genotype in Egypt may be due to a virus mutation in the "a" determinant. [4] The HBV/E genotype was considered to exist mainly in East, Central and West Africa. The fact that many African students from these countries are studying at Al-Azhar University in Tanta City, may, in part, suggest transmission of HBV/E genotype from these African students to Egyptians. Notably, the duration of health care work was significantly longer in those co-infected with HBV/E and D genotypes. This may suggest that HCWs with longer work duration have more extensive exposure, and thus, were more likely to be infected twice.

Hepatitis B vaccination coverage in Egypt is low.^[10] Among 1484 HCWs related to health care facilities from two Egyptian governorates, 158% reported receiving HB vaccination.^[10] Only 8% (**n=45/564*) of HCWs in our study received infantile HB vaccination. Although the anti-HBc sero-positivity (0-0.36 %) is very low among Egyptian general population who received infantile HB vaccination,^[15,21] the anti-HBc was detected in 31 % (**n=14/45*) of HCWs who received HB vaccination in our study; of them, two (**n=2/45*) were infected by HBV/E genotype. This data reflected the high HBV exposure risk among HCWs and infer the waning of protective anti-HBs after adolescent age and suggest the need for HB vaccine boosting.

CONCLUSION

Exposure of Egyptian HCWs to HBV infection in the health care setting is significantly high. Implementation of universal HB vaccination among Egyptian HCWs is needed. National HBV screening and treatment of infected HCWs should be encouraged.

Conflict of Interest

All other authors declare no competing interests.

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How to cite this article: Elmaghloub R, Elbahrawy A, El Didamony G, Elwassief A, Saied Mohammad AG, Alashker A, *et al.* Hepatitis B virus genotype E infection among Egyptian health care workers. J Transl Intern Med 2017; 5: 100-105.