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Immune Response to Hepatitis B Vaccine in Health-Care Workers

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Abstract:

Health care workers (HCWs) constitute a high-risk population of HBV infection. There are limited data on the efficacy of vaccination in HCWs in Egypt.

The aim of this study was to evaluate the immune response to hepatitis B recombinant vaccine in HCWs in our hospital.

Methods: 100 HCWs who completed three doses of intramuscular immunization with recombinant DNA hepatitis B vaccine (Engerix-B) at different time periods during the last 5 years were examined for hepatitis B surface antigen (HBs Ag), anti hepatitis B surface antibodies (anti HBs Abs) and hepatitis B core antibodies (HBc Abs).

Results: 96% of HCWs showed seroconversion (anti HBs ≥ 10 IU/L); 92% good responders (anti HBs > 100 IU/L) and 4% weak responders (anti HBs = 10-100 IU/L). The HBsAg and HBc Ab were never detected among the entire responders. Younger age had higher anti HBs titer than older HCWs. The non responders were 4%; two of them had evidence of chronic hepatitis B infection.

Conclusion: Vaccination against HBV in HCWs in Egypt is cost effective and achieved good response rate. Screening for Hepatitis B infection before vaccination should be considered to detect those with undiagnosed infection.

Key word: Hepatitis B vaccine, Immune response, Egypt, HCWs

Introduction:

Hepatitis B virus (HBV) infection has been a major global cause of morbidity and mortality ***(Kwon and Lee, 2011).*** It is estimated that 350 million patients are chronically infected worldwide and that around half a million die every year from end stage complications of persistent infection ***(Hennig et al., 2008).*** Health care workers (HCWs) are at increased risk of occupational exposure to HBV infection ***(Gholamzadeh and Serati, 2006 and Thakur et al., 2010).***

Fortunately, the currently available hepatitis B Vaccine are extremely safe and have an efficacy of >90 percent. Thus HBV infection can potentially be eradicated through global vaccination ***(Ni et al., 2001 and Chang, 2011).*** In general, no necessity for booster doses for fully vaccinated immunocompetent individuals ***(Van Damme and Van Herck, 2007 and Gabbuti et al., 2007).*** The post vaccination antibody testing and regular testing for antibodies is recommended only to high-risk subjects, especially to health care workers and subjects with immunodeficiency ***(Zannolli and Morgese , 1997 and Pallás Alvarez et al., 2000).***

Although the majority of persons vaccinated against hepatitis B successfully

respond to vaccination, around 5-15% of persons may not respond. "Vaccine non-responder" is a person who does not develop protective surface antibodies after completing two full series of the hepatitis B vaccine and for whom an acute or chronic hepatitis B infection has been ruled out ***(Scolnick et al., 1985).***

The standard anti-HBV vaccination elicits protective anti-HBs levels (above 10 IU/L) in most people ***(Pallás Alvarez et al., 2000).*** According to the anti-HBs produced in response to the recombinant HBV vaccine; low serum level < 10 IU/L is evaluated as lack of protection, serum level =10-100 IU/L corresponds to weak protection and > 100 IU/L is considered as sufficient protection ***(Zannolli and Morgese , 1997, Shatat et al., 2000 and Platkov et al., 2003).***

There are limited data about the rates of post vaccination seroconversion in Egypt among high risk groups especially HCWs who received recombinant hepatitis B vaccination.

Aim of the Study:

To assess immune response to HBV vaccine in HCWs who had completed three doses of hepatitis B recombinant vaccine.

Subjects and Methods:

This study was conducted on HCWs of Gastroenterology department of Ain Shams University Hospitals. An interview was done to enroll subjects who completed three doses of intramuscular hepatitis B recombinant vaccine (Engerix 20 µg offered in infection control unit in the last five years) at 0, 1, 6 months intervals. Subjects with diabetes mellitus, history of infection with HBV or HCV, renal impairment or those on steroids or immunosuppressive therapy were excluded.

Study design:

100 subjects who fulfill the previous conditions were randomly selected to provide a blood sample for anti HBs Ab, HBs Ag and HBc Ab testing. Anti HBs Ab is done to assess the immune response to HBV. HBs Ag and HBc Ab are done to exclude HBV infection. Demographic data and BMI were recorded for all participants. Seroconversion was defined as development of Anti HBs ≥ 10 IU/L.

According to the measured anti-HBs Ab level, participants were classified into three groups:

Non responders: anti-HBsAb level ≤ 10 IU/L .

Weak responders: anti-HBsAb level of 10-100 IU/L .

Good responders: anti - HBsAb level of ≥ 100 IU/L.

All non responders were tested for HBV DNA, HCV Ab in addition to serum transaminases.

An informed consent was obtained from all participants.

Laboratory methods:

Assay of Transaminases:

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by International Federation of Clinical Chemistry (IFCC) method without pyridoxal phosphate, kinetic, U.V at 340 nm on Beckman Synchron CX5 Delta Clinical System (BCKCX5) (Beckman Instruments, Inc., Brea, Ca 92822-8000 USA.). Reagents were obtained from ELITech Group Co. (SEPPIM S.A.S. Zone industrially 61500 SEES France).

HBs Ag and anti HBs Abs detection:

They were tested using HBsAg and anti-HBs EIA DiaSorin S.p.A kits respectively (13040 SALUGGIA (VC), Italy).

Principle:

A typical Direct sandwich ELISA assay in which the antibody, specific for the analyte, is immobilised onto the solid phase; 96-well polystyrene microtitre plate wells.

Technique:

Samples were added to the wells and any corresponding antigen is captured to form an antibody-antigen complex. A wash step is performed to remove any unbound molecules. An antibody labelled with an enzyme is added which binds to form an antibody-antigen-antibody/enzyme conjugate complex and is followed by a wash step. A substrate solution was added, the colour produced change which is proportional to the amount of bound enzyme. Thus samples which do not contain the particular analyte will not form a complex and therefore no colour reaction will take place. Wells that contain samples that do contain the analyte will show a colour change corresponding to the number of individual complexes formed. The colour produced was measured on a spectrophotometer at wavelength 450nm (reference filter 630nm).

Detection of HBV DNA by Real Time Polymerase Chain Reaction (PCR):

Viral DNA was extracted from serum using the QIAamp Min Elute Virus Spin kit (QIAGEN USA) then the extract was added to artus HBV TM PCR Master Mix (17 µl were taken from the artus HBV TM PCR Master Mix (ready to use) and added to 8 µl of the extract to obtain the total volume 25 µl). The reaction tube was put in the Stratagene MX3000P and the thermal cycle of the device was set at: 95 ºC for 10 minutes (one cycle), 95 ºC for 15 seconds and 60 ºC for 1 minute (40 cycles).The detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is called threshold cycle (Ct) and it is proportional to the amount of the specific PCR product.

Statistical Methods:

Data were analyzed using SPSS program version 15. Quantitative data were presented in mean and standard deviation and in case of presence of extreme values the median and range were used. Qualitative data were compared using frequency and percentage.

Results:

1. Demography and BMI:

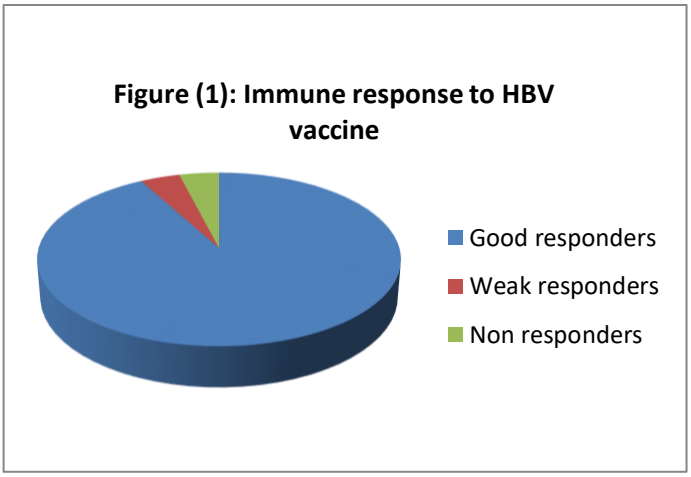
The study included 100 HCWs (doctors and nurses) working in Gastroenterology Department of Ain Shams University. They were 47 males and 53 females with average age 25±6.5 years and BMI 26.5±2.3 kg/m² (table 1)

TABLE 1: Demography and BMI of all participants		
Variables	***Percentage %***	
Demography		
Age in years (Mean ± SD)	25±6.5 (20-46)	
Gender		
males	47/100	47%
females	53/100	53%
BMI (kg/m²) (Mean ± SD)	26.5±2.3	

2. Immune response to HBV vaccine and its relation to age and BMI:

Anti HBs Abs were measured for all subjects who had been vaccinated within the last 5 years by hepatitis B recombinant vaccine; 92% showed good response to the vaccine, 4% had adequate (≥ 10 IU/L) but weak response and 4% were non responders (table 2, figure 1).

TABLE 2: Immune response to HBV vaccine		
Response	**Number of patients**	**HBsAb level (IU/L)** (Mean ± SD)
Good responders	92	855.6±231.6
Weak responders	4	40.4±11.8
Non responders	4	2.4±0.43
Total	**100**	**788.5**±318



The mean age of subjects who showed good response was significantly lower than those who were weak and non responders. The mean BMI was not significantly different between all groups (table 3).

TABLE 3: Relation between the mean age and BMI to the response to HBV vaccine

Variables	Mean ± SD	X^2	P value
Age (years)			
Good responders	24.5±5.9		
Weak responders	32.3±13.4	6.3	≤ 0.05
Non responders	33±7		
BMI (kg/m²)			
Good responders	26.4±2.2		
Weak responders	27.6±2.08	1.1	>0.05
Non responders	27.8±4		

3. Pattern of hepatitis serology :

TABLE 4: Frequency of HBs Ag and HBc Ab in the studied subjects

Groups	Number	HBs Ag		HBc Ab	
		positive	negative	positive	negative
Good responders	92	0	92	0	92
Weak responders	4	0	4	0	4
Non responders	4	1	3	2	2
Total	**100**	**1**	**99**	**2**	**98**

All HCWs who showed seroconversion (good and weak responders) to HBV vaccine were negative for HBs Ag and HBc Ab. In non responders; two subjects were positive to HBc Ab and one of them had HBs Ag detected in his serum (table 4). Both were positive to HBV DNA and had evidence of chronic hepatitis (elevated serum transaminases) (table 5). The other 2 subjects who were non responders had no evidence of hepatitis B or C infection (table 5).

TABLE 5: Serum transaminases and hepatitis serology for non responders.

Non responders	HBsAb IU/L	HBs Ag	HBc Ab	HBV DNA IU/ml	HCV Ab	AST 0-37 IU/ml	ALT 0-42 IU/ml
Case 1	2	-ve	-ve	-ve	-ve	17	19
Case 2	0	-ve	-ve	-ve	-ve	10	14
Case 3	0	+ve	+ve	2400	-ve	62	45
Case 4	0	-ve	+ve	17400	-ve	30	22

Discussion:

Among 100 HCWs included in this study, 96% showed seroconversion in response to the received hepatitis B vaccine. The good responders were 92 (92%) subjects (Their mean anti HBs Abs were 855.6±231.6 IU/L) and the weak responders were 4 (4%) subjects (Their mean anti HBs Abs were 40.4±11.8 IU/L). The non responders to HBV vaccine were only 4 subjects. These results confirm that HBV vaccine is highly effective and its introduction to our infection control program was cost effective.

Many reports from different countries described the efficacy of HBV vaccine which generally ranges between 85% to over 90% ***(Keating and Noble, 2003)***; in Switzerland, seroconversion rate in HCWs was 95% following 3 doses of recombinant HBV vaccine ***(Desgrandchamps and Siegrist, 1998),*** while it was 86% and 86.5% in Pakistan ***(Zeeshan et al., 2007)*** and Israel ***(Platkov et al., 2003)*** respectively. Other epidemiological studies conducted in Iran ***(Gholamzadeh and Serati, 2006)*** and Saudi Arabia ***(Zamani et al., 2011)*** showed response rate to HBV vaccine equal to 87.3% and 82.8% respectively, in both studies not all included subjects had completed 3 doses of the vaccine. Thus, vaccination with the full dose schedule is an important determinant of the response.

We analyzed the age and BMI in the studied population in relation to the pattern of response to HBV vaccine; the mean age of good responders was significantly lower than both weak and non responders (24.5±5.9 years, 32.3±13.2 years and 33±7 years respectively). This agreed with many reports ***(Louther et al., 1998, Yen et al., 2005 and Zeeshan et al., 2007).*** However, ***Zamani et al. 2011*** didn't find age a significant determinant of response to HBV vaccine. BMI in our study was not significantly different between different groups; it was 26.4±2.2 kg/m², 27.6±2.08 kg/m² and 27.8±4 kg/m² for good, weak and non responders respectively. All responders were negative for both HBs Ag and HBc Ab; this confirms that their serum anti HBs were developed in response to the received HBV vaccine. In non responders, two subjects were positive to HBc Ab; one of them was HBs Ag positive and both had HBV DNA in their serum. These two cases had chronic hepatitis B infection which explains the non response to the received vaccine. The other two subjects were free of infection of both hepatitis B and hepatitis C, yet they didn't develop protective antibodies after vaccination.

In conclusion, recombinant hepatitis B vaccine is cost effective and should be administered early (at young age) in full dose to all non vaccinated HCWs. Post vaccination antibodies testing in HCWs is not mandatory, however screening for HBsAg and HBcAb before vaccination is recommended to exclude undiagnosed infection in this high risk group.

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الإستجابة المناعية للتطعيم ضد الفيروس الكبدي (ب) في العاملين بمجال الصحة

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المقدمة: يشكل العاملون بمجال الصحة مجموعة من الأفراد ذو خطورة للعدوى بالفيروس الكبدي ب , وفي مصر لاتتوافر المعلومات عن مدى كفاءة التطعيم ضد هذا الفيروس بين العاملين بمجال الصحة

الهدف من الدراسة: تقييم كفاءة التطعيم ضد الفيروس الكبدي ب بين العاملين بمجال الصحة في مستشفى عين شمس الجامعي.

المرضى و طرق البحث: تضمنت الدراسة 100 عامل بمجال الصحة ممن حصلوا على جرعة كاملة من التطعيم الخاص بالفيروس الكبدي ب خلال الخمس سنوات الأخيرة , وتم للجميع قياس دلالات الفيروس الكبدي ب الأتية: الأجسام المضادة للأنتيجين S و الأنتيجين S والأجسام المضادة للأنتيجين C.

النتائج: لقدوجدت الدراسة أن 96 % ممن حصلوا على جرعة كاملة من التطعيم الخاص بالفيروس الكبدي ب تكونت لديهم أجسام مضادة بشكل مرضي حيث كان 92% ذووا استجابة عالية و 4% ذووا استجابة ضعيفة , ووجد أن 4% لم تتكون لديهم أجسام مضادة (غير مستجيبين) : اثنين منهما يحملان ما يدل على العدوى بفيروس ب (الحمض النووي للفيروس ب بالدم).

الاستنتاج:التطعيم ضد الفيروس الكبدي (ب) للعاملين بمجال الصحة ذو كفاءة عالية وبالتالي فهو مجدي بالنسبة للتكلفة ومن المفضل الإختبار عن وجود أو عدم وجود عدوى قبل التطعيم.