

## Research Article

# Longitudinal Study of Hepatitis B immunization in Healthcare Workers in One Egyptian Center

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**Objective:** We have performed this study to: 1- Evaluate the prevalence of hepatitis B virus surface antigen (HBsAg) and antibody to hepatitis C virus (anti-HCV) in health care workers at start of the study and after 5 years to evaluate the risks of seroconversions 2- Evaluate the rates of antibodies titer in HCWs both after HBV immunization and after 5 years from vaccination.

**Methods:** The study included 302 health care workers in Mansoura University Children hospital, Egypt from December 2009 till February 2015. HCWs receive vaccination for HBsAg and the states of antibodies titer for HBsAg were evaluated after complete vaccination and after 5 years. Also the anti-HCV and HBsAg were determined by immunoassay at start of the study and after 5 years.

**Results:** Ninety percent of HCWs developed anti-HBsAg above 10mIU/ml. The non-responsive of HCWs was 10%, and HCWs with levels between 10-100 mIU/ml were 21.7%. The results of HCV IgG and HBsAg among HCWs at the start of the study were 4.3% and 0.7% respectively. After 5 years, the rates of HCV IgG and HBsAg were 18.2% and 4.4% respectively. The seroconversion rates showed around four folds increase in HCVIgG and six folds increase in HBsAg the increase was highly significant ( $P=0.0001$ ,  $P=0.006$  respectively). The presence of protective antibodies titer after five years for anti-HBs were 59.8%. HCWs that have less 10mIU/ml anti-HBs were 40.3%, HCWs who had levels between 10-100 mIU/ml were 14.5% and HCWs with levels more than 100mIU/ml were 45.3%.

**Conclusions:** The present study highlights an important finding among some Egyptian health care workers. There is good response for hepatitis B vaccination after primary vaccination however, there are some non-responders and hypo responders so the evaluation of antibodies levels after vaccination is important for application of revaccination or/and booster dose application for achieving of utmost protection level. The decline of antibodies level after 5 years is remarkable necessity for the evaluation of health care workers for antibodies levels for booster dose administration. The seroconversion for hepatitis C and B viruses were significant among our health care workers. Strict adherence to infection control guidelines should be implanted.

**Keywords:** Health care workers; Hepatitis B vaccination; Seroconversion

## Introduction

Hepatitis B virus (HBV) is viewed as an important occupational health hazards for health care workers. The routes of infection are contact of mucous membranes and non-intact skin to blood from infected patients [1-3].

WHO statistical analysis demonstrated that around 5.9% of HCWs are every year are in threat of HBV diseases and this outcomes in around 66,000 HBV infections in HCWs overall [4]. Meanwhile health care workers with HBV infections can be considered as a wellspring of hepatitis B infections to patients when proper preventive measures not took after [5].

The most essential danger component for health care workers to acquire hepatitis B virus infection is through exposure prone procedures that involve needles stick injuries and cuts during

operation. This accident is exceptionally basic particularly in health care settings in endemic countries and less developed countries. In any case, stand out third of this episode is accounted to health authorities [4,6-8].

The infection of HBV in HCWs relies on two elements. The first is the status of infected patient with HBV as it is notable that patients with positive HBe antigen (HBeAg) are more infective than those with negative HBeAg as the risk of infection ascends from 1-6% from patients negative to HBeAg to 22-31% from HBeAg positive [9-12].

The prevention of HBV infection in HCWs relies on the practice of universal precautions dealing with counteractive action of transmission of blood borne viral infections like safe needle disposal, wearing gloves during phlebotomy and using eyes goggles [11,12].

The other protective measure is ideal vaccination for HBV surface

antigen (HBsAg) among HCWs. A complete standard vaccination schedule exists in 3 doses that are most normally directed as a 0, 1, and 6-month schedule. A 3-dose course instigates protective antibody concentrations in >90% of healthy adults [13,14]. The base separating of dosages is 4 weeks between doses 1 and 2, 8 weeks between doses 2 and 3, and 16 weeks between doses 1 and 3 [15].

After complete vaccination dosages it is vital to test for advancement of protective sufficient antibodies titer to HBsAg (anti-HBs) as it is accounted for the high frequency of suboptimal protection. Anti-HBs titer beneath 10mIU/ml is considered as suboptimal response to HBsAg vaccination. Anti-HBs levels between 10 and 100mIU/ml are viewed as hypo-responsiveness and levels >100mIU/ml are taken as a high level of immunity [3].

Another hepatitis virus transmitted frequently to HCWs is hepatitis C virus (HCV). It is the commonest cause of chronic hepatitis, liver cirrhosis and liver cancer in Egypt, where 12–15% of the population have HCV antibodies (anti-HCV) [16,17]. HCV prevention among HCWs depends on avoiding exposures [18,19].

Unfortunately, beside the reduced vaccination rates for HBV among HCWs in developing countries, there is also reduced availability about the state of the immunity development after successive immunization and the duration of the lasting immune states. We could not find Egyptian studies about these issues.

Therefore we have performed this prospective study in Mansoura University Children Hospital, Egypt to: 1- Evaluate the prevalence of hepatitis B virus s antigen and anti-hepatitis C virus in health care workers at start of the study and after 5 years to evaluate the risks of seroconversions 2- Evaluate the rates of antibodies titer in HCWs both after HBV immunization and after 5 years from vaccination.

## Materials and Methods

The study included 302 health care workers in Mansoura University Children hospital, Egypt from December 2009 till February 2015. At December 2009 all health care workers were recruited for the study to evaluate the prevalence of HBsAg, hepatitis C virus antibodies IgG. Health care workers negative for HBsAg were subjected to full doses of hepatitis B vaccination (Recombivax HB). The vaccine is transformed in yeast cells. The adult Formulation is without preservative and each 1mL dose contains 10 mcg of hepatitis B surface antigen. It contains the gene for the adw subtype of HBsAg.

After the last dose of vaccination by one month another blood samples were withdrawn for anti-HBs titer evaluation and those with hypo-optimal levels of antibodies were managed according to standard center of disease control guidelines on boosters when antibody levels are found below protective level. In addition, persons found to have anti-HBs levels of <10mIU/mL after administration of three doses on an appropriate schedule were revaccinated.

The study was approved by Mansoura Faculty of Medicine, Egypt and every participant approved to be part of the study and vaccination schedules.

After 5 years, we could recruit 159 of those health care workers. Blood samples were withdrawn for determination of HBsAg, anti-HCV and anti-HBs.

In a reduce the participant bias We have registered records for the vaccinated HCWs in the laboratory for those who have completed the doses and excluded those who were not enrolled for a sufficient period to observe all the doses. The included subjects after 5 years those who were retained in their job in the hospital and have complete recorded data this limit the bias of insufficient follow-up, and if subjects were included else than those at the beginning of the study their outcomes differ from subjects who are included.

For each subject five millitre blood samples were withdrawn. Sera separated and kept frozen at -20°C for virological markers assessment HBsAg, anti-HCV IgG and anti-HBs titer as indication. We used Elecsys system for virological markers studies at the start of the study and after 5 years.

### Principle of the Test for anti-HBs titer

Elecsys uses a sandwich principle: first, a complex is formed with 2 monoclonal HBsAg-specific antibodies, one of which is biotinylated, and the other labeled with a ruthenium complex. After addition of streptavidin-coated microparticles, the complexes bind to the solid phase through interaction of biotin and streptavidin. The mixture is subsequently aspirated into a measuring cell, where application of a voltage induces chemiluminescent emission, which is measured by a photomultiplier. All samples were tested at a 1:400 dilution.

### Principle of the Elecsys Test for HBsAg

Elecsys uses a sandwich principle for detection of HBsAg. This assay is indicated as an aid in the diagnosis of infection with HBV. This assay is also indicated as a donor screening test to detect HBV in serum or plasma specimens from individual human blood donors. It may also be used in testing serum or plasma specimens to screen individual organ donors when specimens are obtained while the donor's heart is still beating.

It has clinical sensitivity 99.9% and specificity 100%. Positive samples for HBsAg were further evaluated for HBV-DNA by PCR and were positive.

### Principle of the Elecsys test for Anti-HCV

Anti-HCV is an *in vitro* diagnostic test for the qualitative detection of antibodies to hepatitis C virus (HCV) in human serum or plasma. This assay is indicated as an aid in the diagnosis of infection with HCV. This assay may also be used to detect antibodies to HCV in serum and plasma specimens to screen donors of cells (excluding blood cells and derivatives), tissues and organs intended for transplantation. The diagnostic sensitivity was found 100% and specificity was 99.71%. Positive and negative controls obtained from the company were used in each run. HCWs with positive anti-HCV were followed out for detection of HCV-RNA by reverse transcriptase polymerase chain reaction; however, results were not mentioned in this study as it was not our goal.

### Statistical analysis

Statistical analysis was performed by the use of SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 16. P values of < 0.05 were considered statistically significant.

## Results

Table 1 summarized the basic demographic data and hepatitis

**Table 1:** Basic Demographic Data and Hepatitis Markers of HCWs (n=302).

Parameter	Value
Age (Years)	34.0± 5.5
<b>Sex</b>	
Males	21 (6.9%)
Females	281 (93.04%)
<b>Job</b>	
Doctors	11 (3.6%)
Nurses	281 (93.04%)
Housekeeping workers	10 (3.3%)
<b>Duration of works</b>	
≤3 years	75 (24.8%)
4-9 Years	156 (51.7%)
≥10 Years	71 (23.5%)
Positive anti-HCV	13 (4.3%)
Positive HBs Ag	2 (0.7%)

**Table 2:** Anti-HBs Response after complete vaccination doses.

Anti-HBs	Number (%)
≤ 10mIU/ml	30 (10%)
≥10mIU/ml	270 (90%)
10-100 mIU/ml	65 (21.7 %)
≥100 mIU/ml	205 (68.3 %)

markers of the HCWs studied. The mean age± SD years of the studied HCWs was 34.0 5.5 the majority were females (93.04%). The principle workers were nurses (93.04%). The duration of works in the hospital was between 4 and nine years among around half of the studied HCWs (51.7%).

The development of anti-HBs was evaluated after complete of the vaccination doses. HCWs who developed anti-HBs above 10mIU/ml were 90%. The non-responsive of HCWs were 10%, and HCWs with levels between 10-100 mIU/ml were 21.7%, (Table 2).

The non-responders were revaccinated and hypo responders were subjected to booster dose of vaccine. Those HCWs were reevaluated after that and the titer was above 100mIU/ml.

The results of HCV IgG and HBsAg among HCWs at the start of the study were 4.3% and 0.7% respectively. While, after 5 years the rates of HCVIgG and HBsAg were 18.2% and 4.4% respectively. The seroconversion rates showed around four folds increase in HCVIgG and seven folds increase in HBsAg the increase was highly significant (P=0.0001, P=0.006 respectively), (Table 3).

It worth noticing that 2 of HCWs whom were positive at the start of the study were included after 5 years among calculated percentage. Both of them were not vaccinated, and they have no antibodies. They have positive HBV-DNA with polymerase chain reaction with normal liver enzymes during the period of the study.

The presence of protective antibodies titer after five years for anti-HBs was 59.8 %. HCWs that have less 10mIU/ml anti-HBs was 40.3%, HCWs who had levels between 10-100 were 14.5% and HCWs with levels more than 100 were 45.3%, (Table 4).

## Discussion

Blood born viral infections are a major threat for HCWs especially in developing countries. In, Egypt, where HCV infections represents an endemic infection there is a need for utmost precautions measures to prevent new incidence of infections with HCV or and HBV

**Table 3:** Hepatitis virological markers of HCWs after 5 years.

Hepatitis markers	After 5 years	Before 5 Years	P
Anti-HCV	29 (18.2%)	13 (4.3%)	P=0.0001
HBsAg	7 (4.4%)	2 (0.7%)	P= 0.006

**Table 4:** Anti-HBs in recruited HCWs after 5 years (n=159).

Titer	Number (%)
≤10 mIU	64 (40.3%)
≥10 mIU/ml	95 (59.8%)

especially among high risk groups like HCWs.

The principle preventing measures are vaccination for HBV and the adequate handling of blood in health settings in hospitals.

The recommendation about the protective level of anti-HBs defines the level equal to or above 10mIU/ml as protective cut of value and to be measured within one to two months after complete vaccination [20,21].

In the present study, the development of anti-HBs was evaluated after complete of the vaccination doses. HCWs who developed anti-HBs above 10mIU/ml were 90%. This finding is online that around 90% of vaccinated adults gives good response with protective levels of antibodies equal to or higher that 10mIU/ml while only 5-10% of the vaccinated subjects have poor response to hepatitis B vaccination [22,23]. The good responders rates among the studied HCWs can be attributed to their young age as the main age± SD was 34.0±5.5 and it has been speculated that vaccination at age younger than 40 years yield good response with effective seroconversion [24,25]. However, the responders among our HCWs were less than that reported previously using Recombivax/HB-Vax II (92.2%) among HCWs group [26]. This may be attributed to difference in the number of the included subjects in the study or different ethnicity that can be associated with different immune response.

HCWs with levels between 10-100 mIU/ml were 21.7% and those with levels below 10mIU/ml were 10%. Previous reports reporting range around 32% of reduced anti-HBs after prolonged duration up to 18 years [27,28].

The immune response to hepatitis B vaccine is influenced by several factors like gender, smoking and obesity. The presence of psychological stress also seems to affect the response to vaccination leading to reduction of immune response [29]. There may be better anti-HBs persistence in males than that of females [30]. This could explain the presence of hypo-responsive subjects in our study as the HCWs were mainly females. Other factors affect also the optimum response for hepatitis B vaccine like the type and the dose of the vaccine and genetic background of the recipients. The numbers of vaccination or cumulative doses have been reported to influence the immunogenicity as primary nonresponders developed protective anti-HBs titers after a booster dose or revaccination [31-33]. The presence of specific polymorphism of the genes of cytokines and cytokine receptors and TLR2 were associated with status of the hepatitis B vaccine-induced protective humoral immune response [34].

Those subjects with anti-HBs below 10mIU/ml had been revaccinated. While subjects with anti-HBs titer between 10-100 mIU/ml, had been given a booster dose as recommended previously.

The estimated duration of the presence of protective antibodies titer for hepatitis B virus differs among different studies. The estimated duration was from 5 years up to 15 years in some studies [35-39].

In the present study, HCWs the presence of protective antibodies titer after five years for anti-HBs i.e was 59.8%. Previous study had detected the seroprotective anti-HBs level to be 47.5% at 6 to 7 years and 39% at 9 year after primary vaccination [35] and similar rate was reported to be 38% among students 18 years after vaccination [40].

However, the noteworthy finding in the present study is that HCWs with anti-HBs titer less 10mIU/ml were 40.3% and HCWs who had anti-HBs titer levels between 10-100 mIU/ml were 14.5%. It is known that in adults the presence of anti-HBs decreases rapidly after one year and slowly after that. A decrease was noticed to a level of <10 mIU/mL in 7%–50% of vaccinated adults within 5 years after vaccination and in 30%–60% within 9–11 years after vaccination [41].

Previous studies in endemic areas for HBV claimed that immunological memory remains intact after 10 years from vaccination and thus protective immunity will be present even after the decline of antibodies titer below 10mIU/ml [42,43]. However, if we can depends upon these studies in the situations of high risk workers like those in health care setting warrant special investigation. Therefore there is a significant role of checking anti-HBs titer regularly at a span of 5 years post HBV vaccination in the HCWs. As HCWs are likely to contact with virally infected body fluids or blood, particularly those residing in countries of high and intermediate endemicity for HBV, they should receive vaccination at their initial entry to their respective training or professional practice. A booster dose should be recommended if anti-HBs titers are low [28].

The results of HCV IgG and HBsAg among HCWs at the start of the study were 4.3% and 0.7% respectively. This is lower rates than that reported in previous study from Egypt where the prevalence of anti-HCV, hepatitis B surface antigen was 16.6%, 1.5% among HCWs [44]. This may be attributed to the difference in the number of the studied HCWs and the difference in their ages as the risk of acquiring HCV and HBV increases with older ages among HCWs.

The distinguished finding in the present study was that the seroconversion rates showed around four folds increase in HCV IgG and six folds increase in HBsAg the increase was highly significant ( $P=0.0001$ ,  $P=0.006$  respectively).

Based on previous survey conducted in Egypt it is estimated that an average of 4.9 needles stick exposures occurs per HCW per year and this results in 24,000 and 8600 Egyptian HCW incidence of HCV and HBV infections respectively based upon mathematical calculation models. Previous study from Egypt measured the incidence of HCV among HCWs to be 0.31% during limited period of study (16.8 months) [45]. The rates of seroconversions for HCV and HBsAg are much higher than that reported in other countries [46-50].

These rates should warrant our attention to provide more education to health care workers about measures reducing the exposure to blood borne viruses, provide adequate personnel protective equipment in our hospitals and encourage the policy of adequate notifying about the needles stick exposures.

The present study highlights an important finding among some

Egyptian health care workers. There is good response for hepatitis B vaccination after primary vaccination however, there are some non-responders and hypo responders so the evaluation of antibodies levels after vaccination is important for application of revaccination or/and booster dose application for achieving of utmost protection level. The decline of antibodies level after 5 years is remarkable necessity for the evaluation of health care workers for antibodies levels for booster dose administration. The seroconversion for hepatitis C and B viruses were significant among our health care workers. Strict adherence to infection control guidelines should be implanted.

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