HOSPITAL PERSONNEL SERO-PROTECTED AGAINST HEPATITIS B VIRUS FOLLOWING AN ACCELERATED VACCINATION PROGRAM

Unyanee Simarugumpai[1], Naesinee Chaiear[1], Pisaln Mairiang[2], Supon Limwattananon[3] and Supaporn Lerdrumpattana[4]

[1]Division of Occupational Medicine, Department of Community Medicine; [2]Department of Medicine, Faculty of Medicine; [3]Department of Social and Administrative Pharmacy, Faculty of Pharmaceutical Sciences; [4]Office of Occupational Health and Safety, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. Accelerated hepatitis B vaccination can rapidly activate immunity against hepatitis B virus (HBV) and reduce rates of post-exposure infection for high-risk healthcare workers. However, no prior Thai studies on accelerated hepatitis B vaccination were published elsewhere. The objective of this study was to measure the proportion of hospital employees who sero-protected against hepatitis B virus following an accelerated vaccination program. From 2014 to 2015, 78 hospital employees were assigned to a three-week vaccination schedule (0, 7 and 21 days). Anti-HBs titers were measured in participants' serum samples two months after the complete vaccination. The proportion of hospital employees sero-protected (anti-HBS positive) was 95.9% (95% CI: 93.7-98.1). Good-responders, hypo-responders and non-responders were 90.54%, 5.41%, and 4.05%, respectively. Geometric mean (GMT) anti-HBs titer was as high as 1,765.71 mIU/ml. This rapid immune responsiveness for accelerated hepatitis B vaccination provided high efficiency for preventing an infection.

Keywords: hepatitis B, vaccine, rapid immunization, hospital employees, health-care workers

INTRODUCTION

Overall, 2,000 million people in the world had hepatitis B virus (HBV) infections, and more than 350 million people had chronic HBV infection (Hamborsky *et al*, 2013). HBV infection causes acute hepatitis, chronic hepatitis, and ends up with fulminant hepatic failure that is a cause of death. More than 80% of hepatocellular carcinoma is caused by HBV infection (Hamborsky *et al*, 2013). The prevalence of HBV infection around the world has declined. Conversely, the prevalence in Southeast Asia has not declined; it is about 5-6%. However, in Thailand the proportion of HBV carriers, both children and young adults, who were born after the universal HB vaccination (1992), was markedly reduced. The figures were as follows: among the age groups 6 months to 5 years, 5-10, 11-20, 21-30, 31-40, 41-50, and >50 years showed 0.10%, 0.29%, 0.69%, 3.12%, 3.78%, 4.67%, and

Correspondence: Naesinee Chaiear, Division of Occupational Medicine, Department of Community Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
Tel: +66 (0) 43 363588; Fax: +66 (0) 43 202488
E-mail: naesinee@kku.ac.th, cnaesi@gmail.com

5.99%, respectively. The seropositivity rate for HBsAg in the post-EPI (Exocrine Pancreatic Insufficiency) group was 0.6%, whereas in the pre-EPI group, it was as high as 4.5% (p<0.001).

Although, there has been a success of universal HB vaccination, some specific occupational groups remain important and require rigorous action for HBV protection (Posuwan, 2016). They are physicians, nurses, dentists, medical students, as well as housekeepers, intravenous drug users, travelers, prisoners, and homosexuals (Connolly and Coreoran, 2013).

The report of Infection Control Unit, Srinagarind Hospital, Khon Kaen University in 2012 showed that healthcare workers (HCW) were disappointed in receiving HBV vaccine, incomplete course of HBV vaccination, and had no blood test after completed vaccination (Bourpoern, Personal communication, 2013). Although HCWs did not have to pay for HBV vaccination, they had poor compliance due to a long duration of the HBV vaccination course (Akande *et al*, 2010). Accelerated HBV vaccination which obtains HBV vaccine at day 0, day 7 and day 21, can increase compliance in HCWs who are at high risk of exposure and desire rapid immunization (Asli *et al*, 2011; Shefer *et al*, 2011).

The aims of our study were: 1) to estimate the proportion of hospital employees who were sero-protected against hepatitis B virus after receiving an accelerated hepatitis B vaccination, 2) to study the potential factors associated with differing levels of anti-HBs titers, and 3) to estimate the differential effectiveness of accelerated hepatitis B vaccination and standard hepatitis B vaccination programs.

MATERIALS AND METHODS

This descriptive study was conducted at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University during 2014-2015. The study population was the pre-placement hospital employees who were enrolled between 2014-2015. The number of participants at the beginning was 78; however, only 74 were tested for hepatitis B virus markers including hepatitis B surface antigen (HBsAg), antibody to surface antigen (anti-HBs) and antibody to core antigen (anti-HBc). Those who were negative for HBsAg, anti-HBs and anti-HBc, were selected for pre-exposure immunization with hepatitis B vaccine. Hepatitis B vaccine (rDNA) 1 ml (20 mcg) (Serum Institute of India, Pune; FDA approved: 2007 Aug 27) was given to an individual's deltoid muscle following the standard operating procedure.

The exclusion criteria were: a history of vaccination for hepatitis B virus, immune deficiency condition, diabetes mellitus, chronic renal failure, age >50 years and history of HBV exposure. The hospital employees were assigned to a three-week vaccination schedule (day 1, day 7 and day 21 after the first dose).

All subjects received three intramuscular injections of recombinant hepatitis vaccine via the deltoid muscle then blood test for anti-HBs titers at 2 months after the 3rd vaccination dose were performed. Electrochemiluminescence Immunoassay; ECLIA (Roche Diagnostic Cobas 6000, Indiannapolis, IN) was used to measure anti HBV titer. In addition, dilution was used when anti-HBs >1,000 mIU/ml and repetition was also applied if anti-HBs were in gray zone (≥8.5 <11.5 mIU/ml).

Statistical analysis

Data was analyzed by SPSS Version 19.0 (IBM, Armonk, NY). Descriptive statistics were used to analyze characteristic of samples such as age, sex, born year,

Table 1
Characteristic of enrolled participants with measurable anti-HBs (*N*=73).

Characteristics	*n* (%)
Gender	
Female	57 (78.1)
Male	16 (21.9)
Date of birth	
Before 1992	61 (83.6)
After 1992	12 (16.4)
Age group (years)	
20-29	53 (72.6)
30-39	11 (15.1)
40-49	9 (12.3)
Job titles	
Patient assistants	21 (28.8)
Registered nurses	14 (19.2)
Nursing assistants	13 (17.8)
Physicians	9 (12.3)
Janitors	5 (6.8)
Public health officers	4 (5.5)
Laundry staff	3 (4.1)
Research assistants	2 (2.7)
Others	6 (8.2)
Occupational risk groups	
High risk	21 (28.8)
Intermediate risk	37 (50.7)
Low risk	15 (20.5)
Body mass index (BMI)	
<18.5	11 (15.1)
18.5-22.9	35 (47.9)
23.00-24.9	14 (19.2)
≥25	13 (17.8)

occupation and risk classification (defined by a likelihood of blood and secretion contact). Proportion of seroprotection in hospital employees were classified by anti-HBs level into three groups; 1) <10 mIU/ml, 2) 10-100 mIU/ml, and 3) >100 mIU/ml. Average of anti-HBs level was presented as geometric mean titer (GMT). Associations between anti-HBs titer and gender, age group, BMI, born year, and compliance were analyzed by one-way ANOVA. Correlation between anti-HBs titer and age/BMI was analyzed by Pearson correlation coefficient. A p-value <0.05 was considered statistically significant.

Table 2
Proportion of subjects who received accelerated hepatitis B vaccination at the appointment.

Receipt of on-time[a] 3 dose series	*N*=73 *n* (%)
3 dose series	53 (72.6)
1st dose	73 (100.0)
2nd dose	54 (74.0)
3rd dose	63 (86.3)

[a]On-time intervals: 1st dose ±7 days, 2nd dose ±4 days, and 3rd dose ±7 days.

Ethical considerations

This study was approved by Khon Kaen University Ethics Committee for Human Research (Ref No: HE 571130). Participants were informed about research methods, advantage/disadvantage of vaccination and side effects. Hospital employees who agreed to be volunteers had to sign informed consents prior to vaccine administration.

RESULTS

Participants were enrolled during 2014-2015. Of the 78 participants, 74 remained for the analysis of proportions, and 73 were included for characteristic describing of anti-HBs titers. The participating hospital employees were Thai nationals; 21.9% (*n*=16) were males and 78.1% (*n*=57) females. The age group with the highest proportion was 20-29 years (72.6%). The majority (83.6%) were born before 1992. Their job titles are listed in Table 1. Among these, 79.5% were classified into the intermediate and high risk of exposure to blood-borne pathogens. Only

Table 3
Geometric mean titer (GMT) of anti-HBs after completion of the accelerated HBV vaccination by factors.

Factor	*n*	GMT	GSD	*p*-value
Gender				0.086
Female	57	1,727.4	7.5	
Male	16	1,909.2	12.5	
Age group (years)				<0.05
20-29	53	2,711.8	8.1	
30-39	11	699.7	3.7	
40-49	9	437.4	10.0	
Date of birth				<0.01
Before 1992	61	1,310.9	7.7	
After 1992	12	8,025.3	6.8	
BMI				0.062
<18.5	11	2,496.9	7.7	
18.5-22.9	35	2,653.5	7.0	
23.00-24.9	14	463.1	8.3	
>25	13	1,310.9	1.0	
Compliance				0.077
On-time	54	1,361.6	7.5	
Delay	19	3,695.5	9.3	

BMI, body mass index; GM, geometric mean; GSD, geometric standard deviation.

17.8% (*n*=13) had their BMI greater than 25 kg/m^2 (Table 1).

Proportion of hospital employees who completed three doses vaccination was 72.6% (*n*=53 subjects). The participants who received first, second and third dose vaccinations on-time were 100%, 74.0%, and 86.3%, respectively (Table 2).

After three doses of vaccination, there was one participant who was negative for anti-HBs (1.35%) and there were two participants (2.7%) whose anti-HBs titer was <10 mIU/ml. Therefore the proportion of hospital employees who revealed sero-protection (anti-HBs positive) after 1 course of vaccination was 95.9% (95% CI: 93.7-98.1). The proportion of anti-HBs titers <10 mIU/ml, 10-100 mIU/ml, 100-1,000 mIU/ml and greater than 1,000 mIU/ml was 4.05% (*n*=3), 5.41% (*n*=4), 35.1% (*n*=26), and 55.4% (*n*=41), respectively. Therefore, the proportion of hospital employees sero-protected (anti-HBS positive) according to the classification of good-responders, hypo-responders and non-responders were 90.54%, 5.41% and 4.05%, respectively.

Overall 95.9% were seroprotected. The geometric mean (GMT) of anti-HBs titer was 1,765.71 mIU/ml and was not different between male (1,909.2 mIU/ml) and female (1,727.4 mIU/ml) (*p*=0.086). Participants who were born after 1992 had a higher anti-HBs titer (8025.3 mIU/ml) than those who were born before 1992 (1,310.9 mIU/ml), (*p*<0.01). Participants aged 20-29 years old revealed a higher anti-HBs titer than those aged >30 years old (*p*<0.05). According to BMI, there was no different anti-HBs titer in each group.

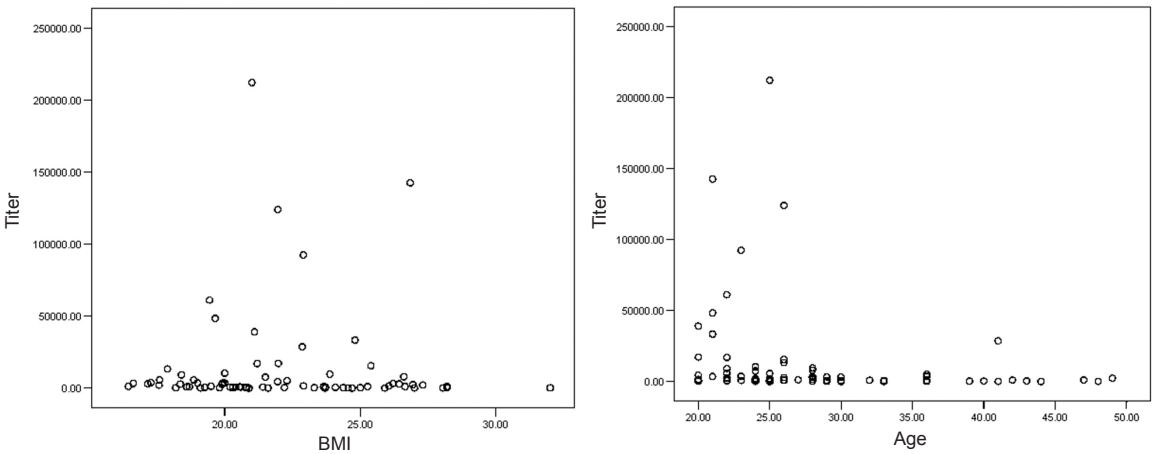

Fig 1–Correlation of BMI or age with anti-HBs titer.

Subjects who received vaccination 'on time' and 'with delay' had no significant difference in anti-HBs level between two groups (p=0.077) (Table 3).

To explore in detail in age and BMI as shown in Fig 1, Pearson correlation showed that no statistically significant difference between anti-HBs titer and BMI (r=0.13) or age(r= 0.201).

DISCUSSION

Accelerated hepatitis B vaccination can rapidly activate immunity against HBV and reduce rates of post-exposure infection for high-risk healthcare workers. Although Thailand is a high-intermediate endemicity area for HBV infection (Posuwan *et al*, 2016) no prior Thai studies on accelerated hepatitis B vaccination were published. The result of the participant characteristics showed that most of them were female (78.1%) which related to the previous studies that showed most of healthcare workers were female (Chanmekha, 2011). Proportion of participants who were born before 1992 was 83.6%, thus this might affect HBV immunization since EPI hepatitis B vaccination was established as Thai public health policy in 1992 (Chokephaibulkit *et al*, 2013). Therefore the majority of participants were not vaccinated against HBV when they were born (Chongsrisawat *et al*, 2006).

The proportion of sero-protection after HBV vaccination course was 95.9% (95% CI: 93.7-98.1). The high proportion was similar to the conventional program therefore rapid immunization program revealed evidence of sufficient protection.

The results of our study were similar to the previous studies reported in USA (Sheffield *et al*, 2011), Iran (Ghadiri *et al*, 2012), Turkey (Saltoglu *et al*, 2003; Tarhan *et al*, 2006) and Germany (Keystone, 2005) where the subjects who had anti-HBs ≥10 mIU/ml after completeness of vaccination was as high as 76.4-96.3%. However, our study showed only three participants (4.05%) had no seroprotection after obtaining three doses of the vaccination. This phenomenon was similar to the reports among healthy population in which about 5-10% showed no seroprotection (Roukens and Visser, 2011; Hamborsky *et al*, 2013; Nashibi *et al*, 2015).

The proportion of sero-protection with high responder (anti-HBs titer ≥100

mIU/ml) was 90.5% (*n*=67) which was much higher than those found in Bangladesh (63.1%) (Chakraborty *et al*, 2011) and in India (68.9%) (Batra *et al*, 2015). These studies did not detect anti-HBs titer within 1-2 months after the completed three doses of vaccination; therefore, the result of sero-protection might have been underestimated.

The GMT of anti-HBs after the accelerated HBV vaccination was 1,765.7 mIU/ml, which was much higher than the studies by Marchou *et al*, (1993) and Ghadiri *et al* (2012). These studies reported levels of 77.6 mIU/ml and 117.0 mIU/ml, respectively. The current study findings may be explained by the fact that the majority of participants were young and had normal BMI in which these factors may affect anti-HBs level responding to HBV vaccination rather than the effect of vaccination booster although GMT anti-HBs of participants born after 1992 was higher than those borne before 1992. In addition, although Khon Kaen was the pilot province for EPI hepatitis B vaccination as earlier as the year 1992, most of them were seronegative prior to starting vaccination program. Therefore, anti-HBs titer following the accelerated HBV vaccination in this study was definitely higher than those previous reports.

According to the factors that may be associated with to anti-HBs level; however, the current study did not find gender differences unlike some studies which revealed females responded to HBV vaccination more than males (Zeeshan *et al*, 2007; Vermeiren *et al*, 2013).

Sero-protection tended to decline with increasing age but there was no statistically significant difference by Pearson correlation coefficient analysis. This finding related to studies by Zeeshan *et al* (2007) and Vermeiren *et al* (2013), which reported no significant difference between age groups. In addition, BMI was also not significantly associated to anti-HBs titer, because the majority of participants were young and had normal BMI. Our study reported a silimilar result to the studies by Chaudhari *et al* (2008) and Nashibi *et al* (2015), which reported no statistically significant difference in BMI. However, Ghadiri *et al* (2012) and Thomas *et al* (2015) reported that anti-HBs titer <1,000 mIU/ml was associated with increasing of BMI. Sheffield *et al* (2011) reported the immune responsed level inverted to grading of BMI because vaccine accumulate in the subcutaneous.

In our study the conlusion of factors which might affect anti-HBV titer could not be drawn, because our study was designed to measure the proportion of the immunized hospital employees. Therefore, the sample size was not calculated for the use of inferential statistics.

According to policy of the Ministry of Public Health (2013), standard HBV vaccination program is recommended for all HCW vaccination schedule (Chokephaibulkit *et al*, 2013). The guideline stated that HCW who were born after 1992 should obtain one dose of HBV vaccine and be tested for anti-HBs titer. In the case of anti-HBs titer is equal or more than 100 mIU/ml, HCW does not need to get 2nd and 3rd doses. However, this is an expert opinion, there is no standard guideline.

Jutavijittum *et al* (2005) and Chongsrisawat *et al* (2006) reported that HBV vaccination was given to all newborns as the policy and covered about 65.7-98.3% of all newborns. Urban children (89.1%) completed vaccination more than rural children (46.9%). Therefore, HCW might not receive HBV vaccine at their birth or

immunity might have declined (Hamborsky *et al*, 2013). Duration of protection relates to anti-HBs titer, so one booster dose might not be enough to protect the high-risk group from post-exposure infection. Therefore, the healthcare workers in high risk groups such as those working in high endemic area, intraverious drug users, multiple partners, immunocompromized host and those who have to undergo hemodialysis, should have a blood test for HBsAg, anti-HBc, anti-HBs before HBV vaccination (Blumberg, 2013; Connolly and Coreoran, 2013; Salisbury and Ramsay, 2013; Macartney and Jelfs, 2015).

In developed countries such as Australia and Ireland, there were recommendation of giving accelerated HBV vaccination to the high-risk groups who required rapid immunization (NIAC, 2013; Department of Health, 2015). According to HBV vaccine recommendation of Thai public health policy, the standard HBV vaccination program was established in either pre-exposure or post-exposure HCW groups. Accelerated HBV vaccination program remained unclear for a recommendation (Chokephaibulkit *et al*, 2013). If accelerated HBV vaccination is useful, proportion of anti-HBs > 10 mIU/ml within one month will be increasing. This is an adventage of accelerated HBV vaccination program, especially in high-risk group who requires rapid immunization. However, the duration of immunized remaining is shorter than those the standard HBV vaccination; therefore, a booster dose at 1 year after accelerated HBV vaccination is necessary.

In summary, our results suggested that an accelerated hepatitis B vaccination schedule on days 0, 7, and 21 provides a protective antibody titer within a shorter time. However, our study has a limitation for extrapolation to nationwide. The accelerated schedule can be used for persons under high risk, such as healthcare workers, who may have direct contact with blood or bloody fluid from patients. We believe the results of our study are crucial for the ongoing evaluation of vaccination strategies and program for healthcare workers in Thailand. For these reasons, we plan an additional follow-up of 5 years after the accelerated vaccination series.

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REFERENCES

Akande TM, Aderibigbe SA. A study of compliance to hepatitis B vaccination among health workers in a Nigerian tertiary health institution. *Niger Hosp Pract* 2010; 5: 13-6.

Asli AAZ, Moghadami M, Zamiri N, *et al*. Vaccination against hepatitis B among prisoners in Iran: accelerated vs. classic vaccination. *Health Policy* 2011; 100: 297-304.

Batra V, Goswami A, Dadhich S, Kothari D, Bhargava N. Hepatitis B immunization in healthcare workers. *Ann Gastroenterol* 2015; 28: 276-80.

Blumberg EA. Guidance for evaluating healthcare personnel for hepatitis B virus protection and for administering post exposure management. *MMWR* 2013; 62: 1-24.

Chakraborty B, Bashar T, Koy K, Noor R, Rahman MM. Persistence of anti-HBs antibody and immunological memory in healthy individuals vaccinated with hepatitis B vaccine. *Stamford J Microbiol* 2011; 1: 37-41.

Chanmekha J. Seroprevalence and risk factor of hepatitis B immunities among Thai health care workers. *Srinagarind Med J* 2011; 26: 112-9.

Chaudhari CN, Bhagat MR, Shah T, Misra RN. Antibody to hepatitis B surface antigen in vaccinated health care workers. *Med J Armed Forces India* 2008; 64: 329-32.

Chokephaibulkit K, Lapphra K, Muangchana C. Textbook of vaccination and immunization. Nonthaburi: Department of Disease Control, Ministry of Public Health, 2013.

Chongsrisawat V, Yoocharoen P, Theamboonlers A, *et al*. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med Int Health* 2006; 11: 1496-502.

Connolly K, Coreoran B, eds. Immunisation guidelines for Ireland [Internet]. Dublin: National Immunisation Advisory Committee, 2013. [Cited 2015 Aug 22]. Available from: http://www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/

Department of Health Australia. The Australian Immunisation handbook. 10th ed. (updated June 2015). Canberra: Department of Health, 2015. [Cited 2015 Aug 22]. Available from: http://www.immunise.health.gov.au/internet/immunise/publishi ng.nsf/Content/Handbook10-home

Ghadiri K, Vaziri S, Afsharian M, *et al*. Comparison of the accelerated and standard vaccination schedules against hepatitis B in healthcare workers. *J Res Med Sci* 2012; 17: 934-7.

Hamborsky J, Kroger A, Wolfe S, eds. Epidemiology and prevention of vaccine-preventable diseases [Internet]. Atlanta: Centers for Disease Control and Prevention, 2013. [Cited 2014 Jul 9]. Available from: http://www.cdc.gov/vaccines/pubs/pinkbook/index.html

Jutavijittum P, Jiviriyawat Y, Yousukh A, *et al*. Evaluation of a hepatitis B vaccination program in Chiang Mai, Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36: 207-12.

Keystone JS. Travel-related hepatitis B: risk factors and prevention using an accelerated vaccination schedule. *Am J Med* 2005; 118(suppl): 63-8.

Macartney K, Jelfs J, eds. The Australian immunisation handbook [Internet]. Canberra: Department Of Health, 2015. [Cited 2015 Aug 22]. Available from: http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook10-home

Marchou B, Picot N, Chavanet P, *et al*. Three-week hepatitis B vaccination provides protection immunity. *Vaccine* 1993; 11: 1383-5.

Nashibi R, Alavi SM, Yousefi F, *et al*. Post-vaccination immunity against hepatitis B virus and predictors for non-responders among medical staff. *Jundishapur J Microbiol* 2015; 8: 1-5.

National Immunisation Advisory Committee (NIAC). Chapter 9: Hepatitis B. In: Immunisation guidelines for Ireland. (Updated 17th September 2015). [Cited 2015 Aug 22]. Available from: www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/chapter9.pdf

Posuwan N, Wanlapakorn N, Sa-nguanmoo P, *et al*. The success of a universal hepatitis B immunization program as part of Thailand's EPI after 22 years' implementation. *PLoS ONE* 2016; 11: e0150499.

Roukens AH, Visser LG. Hepatitis B vaccination strategy in vaccine low and non-responders: a matter of quantity of quality? *Hum Vaccin* 2011; 7: 654-7.

Salisbury D, Ramsay M. Immunization against infectious disease [Internet]. London: Public Health England, 2013. [Cited 2014 Feb 20]. Available from: https://www.gov.uk/government/collections/immunisation-against-infectious-disease-the-green-book

Saltoglu N, Inal AS, Tasova Y, Kandemir O. Comparison of the accelerated and classic vaccination schedules against hepatitis B: three-week hepatitis B vaccination schedule provides immediate and protective immunity. *Ann Clin Microbiol Antimicrob* 2003; 1: 2-10.

Shefer A, Atkinson W, Friedman C, *et al*. Immunization of health-care personnel recommendations of the advisory committee on immunization practices. *MMWR* 2011; 60: 3-8.

Sheffield JS, Hickman A, Tang J, *et al*. Efficacy of an accelerated hepatitis B vaccination program during pregnancy. *Obstet Gynecol* 2011; 117: 1130-5.

Tarhan MO, Aker AI, Sipahi OR, Kardes G, Biberoglu K. Accelerated versus classical hepatitis B virus vaccination programs in healthcare workers accelerated vs. classical HBV vaccination. *Med Sci Monit* 2006; 12: 467-70.

Thomas RJ, Fletcher GJ, Kirupakaran H, *et al*. Prevalence of non-responsiveness to an indigenous recombinant hepatitis B vaccine: a study among South Indian health care workers in a tertiary hospital. *Indian J Med Microbiol* 2015; 33(suppl): 32-6.

Vermeiren AP, Hoebe CJ, Dukers-Muijrers NH. High non-responsiveness of males and the elderly to standard hepatitis B vaccination among a large cohort of healthy employees. *J Clin Virol* 2013; 58: 262-4.

Zeeshan M, Jabeen K, Ali ANA, *et al*. Evaluation of immune response to hepatitis B vaccine in health care workers at a tertiary care hospital in Pakistan: an observational prospective study. *BMC Infect Dis* 2007; 7: 1-6.