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ORIGINAL ARTICLE

What is the current Immune Status of HBV Vaccinees in Health Care Workers and Medical Students in Alexandria University, Egypt?

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ABSTRACT

Background: *Long term immunity of Hepatitis B vaccine, the first vaccine to prevent cancer is a debate.* ***Objective:*** *to assess anti-HBs level in previously vaccinated Health Care Workers (HCWs) and Medical Students (MS). Also, determine the effect of a booster dose on humoral and cell mediated immunity in non seroprotected subjects.* ***Methodology:*** *A cross sectional study included 120 vaccinated HCWs and MS. HBsAg, Anti-HBs level and Interferon γ (IFN-γ) were assessed. Anti-HBs and Interferon γ (IFN-γ) levels were reassessed after a booster dose to non seroprotected subjects.* ***Results****: 90% of HCWs and 23.3% of MS were seroprotected. Boosted HCWs and MS showed significant increase in Geometric Mean Titer (GMT) of anti-HBs level (P=0.028, P<0.001 respectively), while IFN-γ mean concentration level (MCL) increased significantly in MS group only (P=0.01).* ***Conclusion:*** *AntiHBs level decreases significantly over time. Long term memory was found to be still intact after a booster dose.*

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem worldwide and health occupational hazard. It is estimated that 2 billion people worldwide have evidence of past or present infection with HBV, and 258 million are chronic carriers of HBsAg. Chronic HBV infection may cause progressive liver fibrosis, leading to cirrhosis with end-stage liver disease, and a markedly increased risk of hepatocellular carcinoma (HCC). [1]

Vaccination is the most effective measure to reduce the global incidence of HBV.[2] After three intramuscular doses of hepatitis B (HB) vaccine, more than 90% of healthy adults and more than 95% of infants, children, and adolescents develop adequate antibody responses. The level of anti-HBs of 10 mIU/mL is considered to indicate a protective level of immunity. The vaccination schedule includes 3 doses, where the first two doses are separated by no less than 4 weeks, the third dose must be administered at least 8 weeks after the second dose, and at least 16 weeks after the first dose. [3] Vaccination against HBV was integrated in the expanded program of immunization for children in late 1992. According to WHO global immunization data, HB vaccine for infants had been introduced nationwide in 186 countries by the end of 2015. In addition, 101 countries introduced one dose of HB vaccine to newborns within the first 24 hours of life. [4]

The risk of acquiring HBV infection among the health care workers (HCWs) is about 10 times higher than other groups. It was estimated that 5.9% of HCWs worldwide are exposed to HBV annually. Persons beginning employment or training in health care professions are typically required to prove immunity against HBV infection. [5]

Long-term protection by HB vaccination is dependent on the persistence of strong immunologic memory. However, the most important question is how long the protection lasts. Many studies intend to determine HBsAg specific T-cell memory, particularly in vaccine recipients whose serum anti-HBs is less than protective, to make an optimal policy of booster vaccination. [6,7]

The aim of this study was to determine the prevalence of protective anti-HBs level in HBV vaccinees among HCWs and Medical students (MS). Also, to uncover the effect of a booster dose of recombinant HBsAg vaccine in subjects with non protective anti-HBs level with estimation of Interferon gamma (IFN-γ) response in the same group.

METHODOLOGY

Subjects and Methods

Ethical approval:

The study was approved by Medical Research Ethics Committee of AFM. Objectives of the study, procedures, types of information to be obtained, and publication were explained to participants. An informed written consent was obtained from each participant

regarding participation in the study and receiving a booster dose if needed.

Study Subjects

A cross sectional study was carried out during the period of April 2016 to December 2017. The study included two groups; **Group I** Sixty HCWs who previously received 3 doses of recombinant HB vaccine (at 0,1,6 months) as proven by providing their occupational health record. The group was subdivided into 2 subgroups; subgroup Ia (<5 years from third vaccine dose) and subgroup Ib (≥5 years from third vaccine dose). **Group II** included 60 MS born between 1995 and 1996 and were fully vaccinated by the 3 compulsory HB vaccine doses at 2,4,6 months of age as proven by providing their national compulsory vaccination certificate. Exclusion criteria included subjects who didn't complete the 3 doses of the vaccine, subjects with history of chronic illnesses or history of symptomatic clinical hepatitis, subjects with immune disorders or on prolonged therapy with immunosuppressive drugs.

Detailed questionnaire

All participants were interviewed with a pre-designed questionnaire covering age, place of birth, history of allergy to the vaccine, occupational and medical history.

Methods

Stage 1

A venous blood sample (3-5 mL) was withdrawn from each subject aseptically and serum was separated by centrifugation, aliquoted into three labeled sterile eppendorf tubes and stored at -70℃ till processing.

Detection of HBsAg

All serum samples were tested for HBsAg to exclude HBsAg positivity. Qualitative detection of HBsAg was carried out using (Dialab® Double antibody sandwich ELISA for the cut-off determination of HBsAg in human serum or plasma, Austria). [8]

Detection and quantification of anti-HBs titer

Quantitative estimation of anti-HBs was done for participants using (WANTAI® anti-HBs ELISA (Quantitative), Beijing). [9] Plotted in log–log graphic coordinates, the anti-HBs concentration in mIU/ml of each sample was derived from its absorbance using the calibration curve.

Detection and quantification of interferon gamma level

For subjects showing anti-HBs level <10 mIU/ml, quantitative detection of IFN-γ in serum samples was done using ELISA technique (eBioscience® BMS228 human IFN-γ, Austria). [10] Plotted in linear graphic coordinates, the concentration in pg/ml of each sample was derived from its absorbance using the calibration curve.

Stage 2

For subjects having anti-HBs below protective level, a booster dose of recombinant HB vaccine (r-DNA), manufactured by Serum institute of India PVT. LTD was administered.

Detection and quantification of anti-HBs titer

Serum levels of anti-HBs were checked again 4 weeks after the HB vaccine booster.

Detection and quantification of interferon gamma level

Serum levels of IFN-γ were checked again 4 weeks after the HB vaccine booster.

Statistical analysis

Data entry and statistical analysis were done using SPSS software program version 24.0. [11] Anti-HBs Geometric Mean Titer (GMT) was calculated to estimate the central tendency of anti-HBs level in consideration to its skewed distribution. Fisher's Exact and Monte Carlo tests were performed for qualitative data. For comparison between two means, Mann-Whitney U test was used. Wilcoxon Signed Ranks test was used for comparison of means of anti-HBs and interferon gamma before and after HB vaccine booster dose. $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered statistically highly significant.

RESULTS

Demographic data

A total of 120 subjects were enrolled in the study and were divided into 2 groups. **Group I** included 60 HCWs aged 21-58 years with mean age of 32.95±8.73 years. They were 20 nurses, 31 physicians (7 gynecologists, 7 microbiologists, 5 pulmonologists, 3 orthopedists, 3 clinical pathologists, 3 endocrinologists, one dermatologist, one nephrologist, one house officer), 5 lab technicians and 4 hospital workers. Male vs Female percentage was 26.7% vs 73.3%. **Group II** included 60 MS aged 20 years. Male vs Female percentage was 43% vs 57%.

HBsAg testing

All study subjects in both groups were HBsAg negative.

Initial Anti-HBs in Group I

Out of Group I (n=60), 10% (n=6) had initial anti-HBs<10 mIU/ml, 25% (n=15) between 10-99mIU/mL, 50% (n=30) between 100-1000mIU/mL and 15% (n=9) >1000mIU/ml. Analysis revealed 3.3% (1/30) of Subgroup Ia and 16.7% (5/30) of Subgroup Ib had anti-HBs < 10 mIU/ml. GMT of initial anti-HBs was 280.9±5.69 mIU/ml in Subgroup Ia vs 68.08±8.1 mIU/ml in Subgroup Ib. The difference was statistically significant (*P*=0.002). Table (1).

Table 1: Distribution of Group I HCWs according to different levels of initial anti HBs

Initial anti-HBs level (mIU/ml)	Subgroup Ia		Subgroup Ib		Test of significance
	No.	%	No.	%	
0-	1	3.3	5	16.7	Mann-Whitney U **Z=3.14** ***P*=0.002***
10-	5	16.7	10	33.3	
100-	17	56.7	13	43.3	
≥1000	7	23.3	2	6.7	
Total	30	100	30	100	
Minimum-Maximum	0.20 - >1000		1.30 - >1000		
GMT ± SD	280.9 ± 5.69		68.08 ± 8.1		
Median	509.7		97.75		

* Subgroup Ia: HCWs <5 years from third vaccine dose
† Subgroup Ib**:** HCWs ≥5 years from third vaccine dose
‡ GMT±SD: Geometric Mean Titer ± standard deviation
§ Statistically significant p ≤ 0.05

The relation between gender and initial anti-HBs level was assessed in Group I. GMT of initial anti-HBs was 179.87±9.26 among males vs 125.68 ±7.25 mIU/ml among females. The difference was statistically insignificant (Z=1.13, *P*=0.25).

The Age range of 20-29 years at primary vaccination showed the highest percentage of seroprotection (97.4%) among all age groups. The relation between age at primary vaccination and initial anti-HBs level was statistically significant (*P*=0.044). Table (2).

Table 2: Distribution of Group I HCWs according to age at primary vaccination and initial anti HBs level

Age at 1ry vaccination	Anti HBs <10 mIU/ml		Anti HBs ≥10 mIU/ml		Total		Test of significance
	No	%	No	%	No	%	
10-	1	50%	1	50%	2	100%	Monte Carlo test **X^2=8.441** **P=0.044***
20-	1	2.6%	37	97.4%	38	100%	
30-	2	16.7%	10	83.3%	12	100%	
40-50	2	25%	6	75%	8	100%	

*Statistically significant at p ≤ 0.05

Effect of a booster dose of HB vaccine in Group I

After one booster dose of recombinant HB vaccine was administered to HCWs showing anti-HBs <10 mIU/ml (n=6), one subject remained with anti HBs <10 mIU/ml, and belonged to Subgroup Ia. The other 5 subjects who became seroprotected belonged to Subgroup Ib. The GMT of anti-HBs among boosted subjects increased from 1.64± 3.29 to 186.95± 21.42 mIU/ml. This increase was statistically significant (*P*=0.028). Table (3)

IFN-γ level was estimated just before and 4 weeks after booster dose injection. The Mean Concentration Level (MCL) of IFN-γ increased from 23.35 ± 18.75 to 28.04 ± 26.38 pg/ml. The difference was statistically insignificant (*P*=0.225). Table (3)

Table 3: Effect of a booster dose of recombinant HB vaccine on anti HBs and Interferon γ levels of boosted Group I subjects (n=6).

	Anti HBs level (mIU/ml)			Interferon γ level (pg/ml)		
	Before booster dose	After booster dose	Test of significance	Before booster dose	After booster dose	Test of significance
Minimum-Maximum	0.2 - 5.8	0.5 - >1000	Wilcoxon Signed Ranks test **P=0.028***	14.33-61.51	13.42-81.27	Wilcoxon Signed Ranks test **P=0.225**
GMT ± SD	1.64± 3.29	186.95±21.42		23.35±18.75	28.04±26.38	
Median	1.8	1000		15.9	18.47	

*Statistically significant at p ≤ 0.05

Initial Anti-HBs in Group II

Out of Group II subjects (n=60), 76.7% (n=46) had anti-HBs <10 mIU/ml, 20% (n=12) between 10-99 mIU/ml and 3.3% (n=3) >100 mIU/ml.

The relation between gender and initial anti-HBs was assessed in Group II. GMT of anti-HBs was 4.52± 5.52 in males vs 2.42± 3.72 mIU/ml in females, which was statistically insignificant (Z=1.33, *P*=0.18).

Effect of a booster dose of HB vaccine in Group II

After one booster dose was administered to MS showing anti HBs <10 mIU/ml (n=46), only 2.2% (n=1) had anti HBs <10 mIU/ml. The GMT of anti-HBs among Group II boosted subjects increased from 1.66± 2.55 to189.02± 2.93 mIU/ml. This increase was statistically significant (*P*<0.001). Table (4)

The relation between gender and anti-HBs level after booster dose was assessed in Group II. All male subjects (17/17) had anti-HBs ≥ 10 mIU/ml after booster dose, while 3.4% of females (1/29) had anti-HBs <10 mIU/ml. GMT of anti-HBs after booster was 229.7± 1.99 mIU/ml among males vs 168.6± 3.48 mIU/ml among females. However, the difference was statistically insignificant (Z=1.27, *P*=0.20).

IFN-γ level was estimated just before and 4 weeks after booster dose injection. The MCL increased from 18.47 ± 10.58 to 22.84 ± 18.45pg/ml. The difference was statistically significant (*P*=0.01). Table (4)

Table 4: Effect of a booster dose of recombinant HB vaccine on anti HBs and Interferon γ levels of boosted Group II subjects (n=46).

	Anti HBs level (mIU/ml)			Interferon γ level (pg/ml)		
	Before booster dose	**After booster dose**	**Test of significance** Wilcoxon Signed Ranks test **P<0.001***	**Before booster dose**	**After booster dose**	**Test of significance** Wilcoxon Signed Ranks test **P=0.01***
Minimum-Maximum	0.3 – 7.8	2.2 - >1000		12.97-61.51	12.52-90.92	
GMT ± SD	1.66±2.55	189.02±2.93		18.47±10.58	22.84±18.45	
Median	1.95	253.55		14.78	17.04	

* Statistically significant at p ≤ 0.05

Results of HBV vaccines immune status is summarized in flow chart figure.

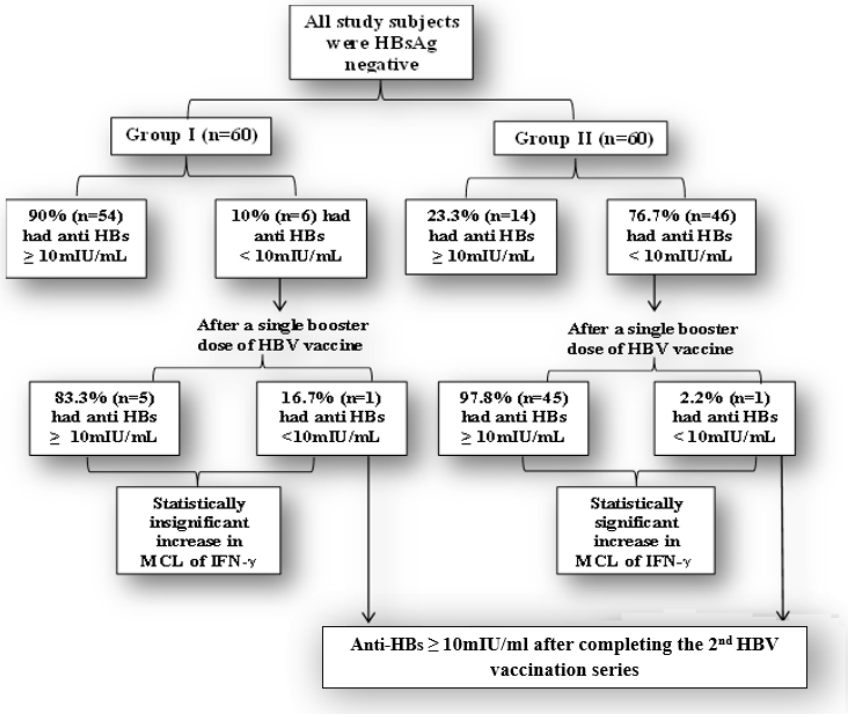

Fig. 1: Study HBV vacinees participants flow chart

DISCUSSION

Post-immunization anti-HBs levels of > 10 mIU/mL defined seroprotection against HBV infection as well as against disease in immunocompetent people. Antibody persistence protects against infection while immune memory protects against disease (acute, chronic hepatitis or carrier state) even after anti-HBs waning. [12] Post vaccination testing is recommended only for those who are at higher risk eg HCWs. An anti-HBs serologic test result of >10mIU/mL indicates immunity. No further routine doses or testing are indicated. If the result is <10 mIU/mL, the vaccine series should be repeated and repeat testing after 1–2 months. [5]

The present study was conducted to assess the current immune status among vaccinated HCWs and MS. Six HCWs (10%) had initial anti-HBs level <10 mIU/ml. Similar findings (11.6%) were detected by Chaudhari S et al. [13] Higher percentages of non seroprotected HCWs were reported by Pavani K et al (2015) and Batra V et al (2015); (16.5% and 30% respectively) [14,15] while Aminian O et al (2016) reported a lower percentage (5.6%).[16]

We found that 3.3% of subgroup Ia (<5years from third dose) vs 16.7% of subgroup Ib (≥5years) had anti-HBs level <10 mIU/ml. Similar findings were detected by Hussein M (2012) where 4% of HCWs vaccinated <5 years ago, showed anti-HBs < 10 mIU/ml. [17] El Sayed ZM et al reported 40.2% of HCWs were non seroprotected > 5 years of HB vaccination. [18] Chaudhari S et al, detected anti-HBs < 10 mIU/ml among 10.2% of HCWs vaccinated < 5 years ago and 14.8% of those vaccinated >5 years ago. [13] GMT of anti-HBs was significantly lower in subgroup Ib than Ia, indicating decline in antibody concentration with time. Similarly, Batra V et al reported significant difference between the mean anti-HBs titer of both subgroups. [15]

In the present study, there was no statistically significant relationship between initial anti-HBs level and sex among HCWs. This was consistent with Chaudhari et al [13] and El Bahnsy et al. [19] However, Pavani et al [14] and Zamani et al [20] reported significantly higher anti-HBs levels among female HCWs compared to males (P = 0.009, P = 0.0001). They claimed that male behavior such as smoking and certain genetic factors may be probable reasons.

We found that HCWs primarily vaccinated at the age of 20-29 years showed the highest percentage of protective anti-HBs (97.4%) compared to other age groups. This was statistically significant (P=0.044). This was also reported by Chaudhari et al [13] and El Bahnasy et al [19] (92.8% and 68.7% respectively).

After one booster dose was administered, only one HCW (1/6, 16.7%) still showed anti-HBs <10 mIU/ml. Similar results (16.6%) were reported by Lopes et al.[21] A lower percentage (12.8%) was detected by Joukar et al.[22]

Immune status was also assessed among infantile compulsory vaccinated MS 20 years ago. Out of 60 students, 23.3% (n=14) had anti-HBs ≥10 mIU/ml. Different follow up post vaccination results were detected by several studies in different countries, even in those with the same vaccination schedule and the same duration since primary vaccination. Salama I et al [23], Jamebozorgi et al [24] and Ciampini et al [25] reported higher percentages (30.5%, 37% and 59.3% respectively) after 15-20 years from compulsory vaccination. A lower prevalence of seroprotection was detected by Eladawy et al [26] and Spradling et al [27] (8.9% and 14% respectively).

In our study, there was no significant difference in initial anti-HBs level among MS as regards the sex. This was consistent with Salama et al[23] and Dumaidi et al.[28]

After one booster dose administration, only one MS (n=1/46, 2.2%) had anti HBs <10 mIU/ml. Similar result (2.9%) was detected by Jamebozorgi et al.[23] Higher percentages were detected by Ciampini et al [25] and Spradling et al [27] (10% and 35.9% respectively). In relation between gender and anti-HBs among MS after booster dose, GMT of anti-HBs after booster dose was higher in males than females but the difference was statistically insignificant. Jamebozorgi et al [24] found higher GMT of anti-HBs in females as compared to males after booster, but the difference was also statistically insignificant.

During recent years, several studies have attempted to detect and measure HBsAg-specific T cells and B-cells immunologic responses among vaccinated individuals with different results. [7] Quantification of IFN-γ expression by T cells is a well-established test for assessing cell-mediated immune responses. Several IFN-γ detection methods have been developed. In vitro assays include the enzyme-linked immunosorbent assay (ELISA), ELISPOT assay and intracellular cytokine staining (ICS) assay. In vivo assays include estimation of IFN-γ in human serum after HB vaccination using ELISA.[29] We preferred to assay the in vivo response to a booster dose of HB vaccine on both humoral and cell mediated immune response. To increase the accuracy of study method, we compared the post booster cytokine levels with those before booster dose.

In the present study, for the 6 boosted HCWs, the IFN-γ MCL increased but it was statistically insignificant (P=0.225). Shooshtari M (2015) et al reported 69.2% positive response after using in vitro ELISA. [29] Regarding the 46 boosted students, The IFN-γ MCL increase was statistically significant (P=0.01). Saffar et al detected IFN-γ increase in 80% of his study group. [10]

CONCLUSION

AntiHBs level decreases significantly in MS and HCW vaccinated 20 years and ≥5 years ago respectively. Long term memory was found to be still intact after a booster dose. Long term studies for more than 30 years are important to assess lifelong immune memory and the need of a booster dose in high risk group.

Limitations and recommendations

As non responders were not detected in our study, we were not able to evaluate the association of decreased immune response with risk factors like smoking, nutritional status, and genetic factors which can contribute to reduced immune response. National surveillance systems to assess the prevalence of HBV infection among HCW, monitoring injection safety practices, occupational exposure and postexposure management, databases identifying HB vaccine responders and nonresponders, studies determining efficacy and cost-effectiveness of antiviral postexposure prophylaxis are needed.

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Authors contribution: This work was carried out in collaboration between all authors. Malak A. Abou Khatwa, May M. Raouf conceived and designed the experiments. Nancy M. Ouf performed the laboratory work, performed the statistical analysis. May M. Raouf, Nancy M. Ouf managed literature searches and wrote the first draft of the manuscript. Malak A. Abou Khatwa, May M. Raouf, Nancy M. Ouf jointly developed the structure and arguments for the paper. All authors reviewed and approved the final manuscript.

REFERENCES

1. HBV- World Health Organization (WHO). Available from URL: http://www.who.int/news-room/fact-sheets/detail/hepatitis-b/;2017. Accessed 11 May 2017
2. World Health Organization (WHO). Hepatitis B vaccines: WHO position paper – weekly epidemiological report. 2107 VOL 92, NO 27; 369-392. Available from URL: http://www.who.int/wer/2017/en/. Accessed 11 August 2017
3. Centers for Disease Control and Prevention (CDC). Hepatitis B. In: Epidemiology and Prevention of Vaccine-Preventable Diseases "The Pink Book". Available from URL: https://www.cdc.gov/vaccines/pubs/pinkbook/hepb.html/;2017. Accessed 11 August 2017.
4. World Health Organization (WHO). Immunization coverage fact sheet. 2018 Available from URL: http://www.who.int/news-room/fact-sheets/detail/immunization-coverage/;2018. Accessed 26 April 2018.
5. World Health Organization (WHO). Immunization of health care workers. Available fromURL:

   http://www.who.int/immunization/policy/Immunization_routine_table4.pdf/;2018 Accessed 21 May 2018.
6. Spradling P, Xing J, Williams R, Faleafaga Y, Dulski T. Immunity to hepatitis B virus (HBV) infection two decades after implementation of universal infant HBV vaccination: Association of detectable residual antibodies and response to a single HBV challenge dose. *Clin Vaccine Immunol*. 2013; 20(4): 559–561.
7. Saffar H, Saffar M, Ajami A, Khalilian A, Esfandabad K. Long-Term T-Cell-mediated immunologic memory to hepatitis B vaccine in young adults following neonatal vaccination. *Hepat Mon*. 2014; 14(9): e22223.
8. Dialab® Double antibody sandwich ELISA for the cut-off determination of HBsAg in human serum or plasma, Austria. Available from URL:http://www.dialab.at/nc/en/downloads/downloadCategory/inserts.
9. Wantai Hepatitis B Virus Diagnostics. WANTAI anti-HBs ELISA (Quantitative). Available from URL:http://www.ystwt.cn/IFU/HBV/anti-HBs(Q).pdf.
10. Human IFN gamma Platinum ELISA BMS228 for quantitative detection of human IFN gamma. Available from URL: http://tools.thermofisher.com/content/sfs/manuals/228.pdf.
11. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 24.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning. 2015
12. Leuridan E, Van Damme P. Hepatitis B and the Need for a Booster Dose. *Clin Infect Dis.* 2011; 53(1), 68–75. https://doi.org/10.1093/cid/cir270
13. Chaudhari S, Bhagat C, Shah T, Misra S. Antibody to hepatitis B surface antigen in vaccinated health care workers. *MJAFI*. 2008; 64(4): 329-332.

14. Pavani K, Srinivas MS, Samhitha M, Vinayaraj EV, Dass S. Immune Response to hepatitis B vaccine in health care workers in tertiary care hospital in South India. *IJ Microbiol Res*. 2015; 2(4):210-213.
15. Batra V, Goswami A, Dadhich S, Kothari D, Bhargava N. Hepatitis B immunization in healthcare workers. *Ann Gastroenterol*. 2015; 28(2): 276–280.
16. Aminian O, Saidi M, Haghighi K, Izadi N. Coverage and Long-Term Immunogenicity of Hepatitis B Vaccine in Healthcare Workers. *Arch Clin Infect Dis*. 2016: e35758.
17. Hussein MM. Immune Response to Hepatitis B Vaccine in health-care workers. *EJHM*. 2012;49: 596– 603.
18. El Sayed ZM, El Razek HMA. Longitudinal Study of hepatitis B immunization in healthcare workers in one Egyptian center. *J Immun Res*. 2016; 3(1): 1026.
19. El Bahnasy RE, Abu Salem ME, El Shazly HM, Morad WS. Predictors of poor response to the hepatitis B vaccine among healthcare workers at the National Liver Institute Hospital. *Menoufia Med J*. 2016; 29:131-135.
20. Zamani F, Fallahian F, Hashemi F, Shamsaei Z, Alavian SM. Immune response to hepatitis B vaccine in health care workers. *Saudi J Kidney Dis Transpl*. 2011; 22:179-184.
21. Lopes M, Sartori A, Souza T, Mascheretti M, Chaves T. Hepatitis B revaccination for healthcare workers who are anti-HBs-negative after receiving a primary vaccination series. *Rev Soc Bras Med Trop*. 2012; 45(5): 639-642.
22. Joukar F, Ghanaei F, Naghipour M, Asgharnezhad M. Immune Responses to single-dose versus double-dose hepatitis B vaccines in healthcare workers not responding to the primary vaccine series: A randomized clinical trial. *Hepat Mon*. 2016; 16(2): e32799.
23. Salama I, Sami S, Said Z, El-Sayed M, El Etreby L. Effectiveness of hepatitis B virus vaccination program in Egypt: Multicenter national project. *World J Hepatol*. 2015; 7(22): 2418-2426.
24. Jamebozorgi M, Keshavarz J, Nemati M, Hossainabad S, Rezayati M. The persistence of anti-HBs antibody and anamnestic response 20 years after primary vaccination with recombinant hepatitis B vaccine at infancy. *Hum Vaccin Immunother*. 2014; 10(12): 3731–3736.
25. Ciampini S, Coppeta L, Capanna A, Gervasi G, Giambini I. Hepatitis B virus protection 20 years post-vaccination in healthcare students: An observational retrospective study at the University of Rome Tor Vergata. *Ann Virol Res*. 2016; 2(1): 1011.
26. Eladawy M, Gamal A, Fouad A, ElFaramawy A. Hepatitis B Virus Vaccine immune response in Egyptian children 15-17 years after primary immunization; should we provide a booster dose? *EJPAI*. 2015; 13(2):45-48.
27. Spradling P, Xing J, Williams R, Faleafaga Y, Dulski T. Immunity to Hepatitis B virus (HBV) infection two decades after implementation of universal infant HBV vaccination: Association of detectable residual antibodies and response to a single HBV challenge dose. *Clin Vaccine Immunol*. 2013; 20(4): 559–561.
28. Dumaidi K, Al-Jawabreh A. Persistence of anti-HBs among Palestinian medical students after 18 - 22 years of vaccination: A cross-sectional study. *Hepat Mon*. 2015; 15(11): e29325.
29. Shooshtari M, Makvandi M, Rasti M, Neisi N, Rastegarvand N. Evaluation of hepatitis B surface antibody and specific gamma interferon response in health care workers after vaccination. *Jundishapur J Microbiol*. 2015; 8(1): e13801.