



ANTI HEPATITIS B TITRES IN HEALTHCARE WORKERS FOLLOWING HEPATITIS B IMMUNIZATION IN A TERTIARY CARE HOSPITAL

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Conflicts of Interest: Nil

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Abstract:

Introduction: Hepatitis B virus (HBV) has long been recognized as an occupational hazard for health-care workers (HCW), including trainees [1]. Testing for postvaccination antibody titres (anti-HBs) identifies vaccine non-responders and hence guides the need for revaccination, additional testing for chronic HBV infection, and counseling [1]. In the current study, retrospective analysis of the anti-HBs titres of health care workers was performed to assess their response to hepatitis B vaccination and the variations with respect to age and time.

Methods: A retrospective cross sectional data analysis of post vaccination serology of health care workers following completion of their Hepatitis B immunization schedule (0,1 and 6 months) as part of their annual health checkup was performed by determining anti HBs titres quantitatively using Abbot Architect Chemiluminescence assay.

Results: The age range of the study participants was 18-60 years. Among the 239 HCWs, 96.7% ($n = 231$) had protective immunity to hepatitis B. There was no significant difference in antibody response between males and females ($P = 0.314$). There was a decline in immune response as the age was increasing ($P = 0.008$). The results of the study found a significant decline in the immune response with time ($P = 0.04$)

Conclusion: Post vaccination immunity to hepatitis B was 96.7% in HCW and was found to correlate with the standard rates. The immunity was found to decrease with increasing age and over a period of time. More studies are required regionally and across the country to determine varied response in terms of post vaccination serology. Further research is required for improving the immunogenicity of existing vaccines and to sustain it over a longer period ensuring better occupational safety in developing countries. The importance of estimating anti HBs titres post vaccination is important for administering booster doses protecting the HCWs from occupational exposures.

Keywords: Anti-hepatitis B virus surface antigen levels, Health care workers, Hepatitis B vaccine, Hepatitis B virus.

Introduction

Hepatitis B virus (HBV) is an occupational risk for health-care workers (HCW), including students and trainees [1]. The virus has drawn importance as it can remain infectious for prolonged periods on environmental surfaces and can also be transmitted even in the absence of visible blood [1]

Hepatitis B can be transmitted from patients to healthcare workers (HCWs) and from HCWs to patients [2]. As per WHO, approximately 5.9% of HCWs each year are exposed to blood-borne HBV infections accounting to about 66,000 HBV infections in HCWs across the world.[2]

Among people who are not vaccinated, the risk of acquiring infection ranges from 6–30% following single exposure [3].

Vaccinating with HBV surface antigen (HBsAg) vaccine can prevent developing Hepatitis B infection.[2] First vaccine for Hepatitis B (Heptavax) was made available since 1982 which was derived from plasma and later from 1984 as recombinant vaccine [4,5].

The vaccine has been demonstrated to offer protection against acute and chronic disease among vaccine responders who are immunocompetent.[6] Currently, two genetically engineered vaccines, made by inserting the gene for HBsAg into the yeast *Saccharomyces* and

harvesting the HBsAg produced are available worldwide.[7] The recommended dosing schedule approved by the regulatory authorities is zero, one and 6 months and an accelerated regimen (zero, one and 2 months, with a booster dose of vaccine at 12 months has also been accepted .[7] When routine immunization is considered , the '0, 1 and 6 months' schedule is less costly and provides excellent antibody titres and long duration of protection. [8]

The centre for disease control has recommended in 1997 that all HCWs should receive a complete course of hepatitis B vaccination administered intramuscularly at 0, 1, and 6 months.[1] Protective antibody titres are achieved in almost all recipients by 3 months.[8] Following 3 dose series, the protective antibody levels are obtained by more than 95% of young healthy adults. However, the level of antibody response reduces with age (< 90% response at 40 years and 75% response at 60 years). Certain factors reduce the immune response such as obesity, chronic diseases, smoking and malnutrition [5].

Testing for protective antibody by determining anti-HBs titres becomes important as some individuals do not develop sufficient levels of antibodies against HBsAg. Non response to vaccination is indicated by anti-HBs titre less than 10 mIU/ml, hyporesponse when between 10 and 100 mIU/ml and high level of immunity when more than 100 mIU/ml after completion of 3 doses of vaccination. Titres more than 10 mIU/ml at any point of time following vaccination provides protection against infection and is considered as a marker of sustained immunity . [7]

Factors like smoking, obesity, aging, chronic medical conditions, male sex, genetic factors and immune-suppression have been considered for a decreased immune response.[9][10]

The current study is conducted to determine the anti-HBs titres among HCWs as part of the annual health checkup in response to Hepatitis B vaccination after completion of the course and the impact of post vaccination duration and increasing age on the immune response for administering booster doses.

MATERIALS AND METHODS

A retrospective cross sectional study was conducted at Institute of Nephro-Urology, a tertiary care referral hospital, Victoria hospital complex, Bangalore for a period of 3 months between December 2019 to Feb 2020, using the data of anti-HBs titers mounted by the health care workers following completion of Hepatitis vaccine schedule as part of their annual health checkup.

INCLUSION CRITERIA:

All HCWs, who have received the complete standard course of intramuscular HBsAg vaccination which is three doses at 0, 1 and 6 months.

EXCLUSION CRITERIA:

Known HBsAg positive HCWs.

The data of a total of 239 HCWs were retrospectively analyzed. A categorical analysis was performed from the data obtained for age, gender and post vaccination duration on comparison with the anti-HBs titres.

Determination of anti-HBs titres:

With strict adherence to aseptic precautions, 3 ml of venous blood was collected in a compatible vacutainer from each member of the study population. Blood sample was centrifuged at 3000 rpm for 5 minutes at room temperature and the serum thus separated was stored at -80°C until further analysis. From the collected serum samples anti- HBs antibody titres were measured quantitatively using an Abbot Architect chemiluminescence assay as per the standard operating procedure provided by the manufacturer.

STATISTICAL ANALYSIS:

For 99% confidence interval and an allowable error of 5%, the sample size required is 77. As our hospital is a tertiary care center, we have taken a total of 239 HCWs who represent our study population. Data was analyzed for age, gender and duration since last dose against Anti HBs titres using SPSS 30 software. Chi square test cross tabulation was used for comparison. P value was of <0.05 was considered significant.

RESULTS:

The study population comprised 166 (69.4%) females and 73 (30.54%) males. All the 239 participants had received 3 doses of vaccination. 95 (39.74%) participants had taken booster doses in addition.

Overall, 231(96.7%) of the vaccinated HCWs developed protective immunity to hepatitis B. Of these 49 (20.5%) had an anti-HBs titre between 10 and 100 mIU/ml and the rest 182 (76.1%) had an anti-HBs titre of >100 mIU/ml. 8 (3.3%) of the vaccinated HCWs had insufficient anti-HBs response [Table 1]. There was a decline in immune response as the age was increasing. ($P = 0.008$) [Table 2]. There was no significant difference in antibody response between males and females ($P = 0.314$) [Table 3]. The duration between the last dose of vaccination and the time of assessment of titres ranged from 6 months to 14 years. It was evident that immunity against HBV had reduced significantly overtime ($P = 0.04$) [Table 4]. The anti-HBs

response improved following booster dose. ($P = 0.03$) [Table 5].

TABLE 1: DISTRIBUTION OF ANIT-HBS TITRES IN STUDY POPULATION

<10mIU	10-100mIU	>100mIU	TOTAL
8	49	182	239
3.3%	20.5%	76.1%	100%

TABLE 2: ASSOCIATION BETWEEN AGE AND ANTI-HBS TITRES

AGE (IN YEARS)	<10mIU	10-100mIU	>100mIU	Pearson square value	Chi P value
<30 YEARS	0	23	98	13.95	0.008
30-35 YEARS	0	6	58		
>35 YEARS	8	20	26		

TABLE 3: ASSOCIATION BETWEEN GENDER AND ANTI-HBS TITRES

	<10	10-100	>100	Pearson Chi-square value	P value
FEMALE	7	31	128	2	0.314
MALE	1	18	54		
TOTAL	8	49	182		

TABLE 4: ASSOCIATION BETWEEN DURATION AND ANTI-HBS TITRES

	<10 mIU	10-100 mIU	>100 mIU	Pearson value	Chi-square P value
<5 years	2	22	98	5.6	0.04
>5 years	6	27	84		

TABLE 5: ASSOCIATION BETWEEN BOOSTER DOSES AND ANTI-HBS TITRES

ONE BOOSTER DOSE	<10 mIU	U	>100mIU	Pearson Chi-square Value	P value
YES	0	17	78	5.8	0.03
NO	8	38	98		

DISCUSSION

The age range of the study participants was 18-60 years. There was no significant difference with respect to antibody response between males and females ($P = 0.314$). There was a decline in immune response with increasing age ($P=0.008$). As the duration since the last dose of vaccine increased, the antibody response reduced ($P \leq 0.04$)

Among the 239 staffs, 96.6% ($n = 231$) had protective immunity to hepatitis B. Study by Varshochi et al [11] and Thomas et al [12] had 98.54% and 98.89% response rate which is compatible with our study. Studies by

Chakrabarthy et al [13] and Nagamani et al [14] had 100% response rates. In our study, slight decrease in number of staffs with protective immunity comparatively could be due many of the staffs with more than 5 years of post-vaccination duration.

We found that the antibody titres reduced with increasing age. This result is similar to a study conducted by Zeeshan et al [15]. They studied 666 employees from Aga Khan University and found that the response reduced with increasing age. Theoretically, lymphocyte proliferation reduces with age.

There was no association between gender and the anti-Hbs antibody response in our study. Perara et al [16] studied 258 medical students in Colombo. They also found no association between gender and antibody response, which is compatible with our study. Study by Zeeshan et al [15], showed poor response in males compared to females. The reduced response in males could be attributed to smoking and genetic factors.

HCWs with longer duration from the last dose of vaccination had reduced antibody titres in our study. This result is compatible with a study by Whittle et al [17]. The reduced antibody response with increasing duration may be due to decreased exposure to the antigen.

Immune response improved significantly after booster doses. This result is similar to the study by Alimonos et al. [18] Booster dose will further stimulate vaccine induced immune response.

The limitation of our study was that we did not study other factors which could have caused reduced immune response such as smoking, genetics, route of administration, obesity, malnutrition. Also, we have not tested hepatitis B core antibody levels due to limited resources. Hence there is also a possibility of HBsAg infection which could have caused low antibody response.

Post vaccination anti HBs antibody testing is recommended for all healthcare workers, public safety workers, patients on maintenance hemodialysis, immunocompromised patients such as those with HIV infection. Also it is recommended for all sex or needle sharing partners of Hepatitis B infection. [19][20]

Our study provides a base for future epidemiological studies in India. It is required for the safety of all HCWs, who are the lifeline of this country. Also, it is cost effective compared to post exposure prophylaxis with immunoglobulins.

Lot of research is ongoing to develop a vaccine with increased immune response which can be uniformly administered to all individuals irrespective of the age, gender, comorbidities and so on. Incorporating Pre S1 and

Pre S2 protein in the vaccine and use of a more immunogenic adjuvants are some of the ways to increase the antibody response. [21] More research is needed on increasing the number of doses or changing the route of administration of the vaccine. Few other approaches under investigation are the use of plasmid DNA encoding HBsAg and the use of HBsAg pulsed dendritic cells. [22]

Conclusion

The seroconversion rate after the standard doses of vaccination was approximately similar to other international studies with respect to age, gender and duration since the last dose. It is important to check the antibody titres 6 to 8 weeks post vaccination in all healthcare workers for their safety and also to reduce the rate of transmission of the infection. It is also a very cost effective approach in developing countries like India.

References:

1. CDC Guidance for Evaluating Health-Care Personnel for Hepatitis B Virus Protection and for Administering Postexposure Management. Recommendations and Reports .2013;62:10
2. Prüss-Ustün A, Rapiti E, Hutin Y. Estimation of the global burden of disease attributable to contaminated sharps injuries among health-care workers. *Am J Ind Med* 2005;48:482-90
3. Sahana HV, Sarala N, Prasad SR. Decrease in Anti-HBs Antibodies over Time in Medical Students and Healthcare Workers after Hepatitis B Vaccination. *Biomed Res Int.* 2017;2017:1327492. doi: 10.1155/2017/1327492.
4. E. Mast, F. Mahoney, M. Kane, and H. Margolis, "Hepatitis B vaccine," in *Vaccines*, Plotkin S. A., Orenstein W. A., and Offit P. A., Eds., pp. 299–337, Elsevier, Philadelphia, Pennsylvania, Pa, USA, 4th edition, 2004.
5. Michael John .Hepatitis B Immunization and Postimmunization Serology. *J Can Dent Assoc* 2000; 66:551-2.
6. Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. *Clin Infect Dis* 2011;53:68-75
7. Jilg W, Schmidt M, Deinhardt F. Vaccination against hepatitis B: comparison of three different vaccination schedules. *J Infect Dis* 1989; 160:766-9.
8. McMahon BJ, Dentinger CM, Bruden D, Zanis C, Peters H, Hurlburt D, et al. Antibody levels and protection after hepatitis B vaccine: Results of a 22-year follow-up study and response to a booster dose. *J Infect Dis* 2009;200:1390-6
9. Averhoff F, Mahoney F, Coleman P, Schatz G, Hurwitz E, Margolis H, et al. Immunogenicity of hepatitis B vaccines. Implications for persons at occupational risk of hepatitis B virus infection. *Am J Prev Med* 1998;15:1-8
10. Shaw FE Jr., Guess HA, Roets JM, Mohr FE, Coleman PJ, Mandel EJ, et al. Effect of anatomic injection site, age and smoking on the immune response to hepatitis B vaccination. *Vaccine* 1989;7:425-30.
11. Varshochi M, Mahmodian R. Infectious diseases and tropical medicine research center, department of infection control nurse, cardiovascular research center, faculty of medicine, Tabriz University of medical sciences, Tabriz, Iran. *Am J Immunol* 2011;7:12-6
12. Thomas RJ, Fletcher GJ, Kirupakaran H, Chacko MP, Thenmozhi S, Eapen CE, et al. Prevalence of nonresponsiveness to an indigenous recombinant hepatitis B vaccine: A study among South Indian health care workers in a tertiary hospital. *J Med Microbiol* 2015;33 Suppl S1:32-6.
13. Chakraborty B, Bashar T, Roy 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;1:2074-5356
14. Nagamani R, Naidu A, Jayanthi SR. Study of hepatitis b surface antibody titres in female medical students post vaccination. *Int J Curr Microbiol Appl Sci* 2017;6:1053-9
15. Zeeshan M, Jabeen K, Ali AN, Ali AW, Farooqui SZ, Mehraj V, 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;7:120
16. Perera J, Perera B, Gamage S. Seroconversion after hepatitis B vaccination in healthy young adults, and the effect of a booster dose. *Ceylon Med J* 2002;47:6-8
17. Whittle HC, Maine N, Pilkington J, Mendy M, Fortuin M, Bunn J, et al. Long-term efficacy of continuing hepatitis B vaccination in infancy in two Gambian villages. *Lancet* 1995;345:1089-92
18. Alimonos K, Nafziger AN, Murray J, Bertino JS Jr. Predictions of response to hepatitis B vaccine in health care workers: whose titers of antibody to hepatitis B surface antigen should be determined after a three-dose series, and what are the implications in terms of cost-effectiveness? *Clin Infect Dis* 1998; 26:566-71
19. Ali NS, Jamal K, Qureshi R: Hepatitis B vaccination status and identification of risk factors for hepatitis B in health care worker. *J Coll Physicians Surg Pak* 2005, 15(5):257-260.
20. Mujeeb SA, Khatri Y, Khanani R: Frequency of parenteral exposure and Seroprevalence of HBV, HCV and HIV among operation room personnel. *J Hosp Infect* 1998, 38:133-7
21. Rottinghaus ST, Poland GA, Jacobson RM, Barr LJ, Roy MJ. Hepatitis B DNA vaccine induces protective antibody responses in human non-responders to conventional vaccination. *Vaccine* 2003;21:4604-8.
22. Kim JJ, Ayyavoo V, Bagarazzi ML, Chattergoon MA, Dang K, Wang B, et al. *In vivo* engineering of a cellular immune response by coadministration of IL-12 expression vector with a DNA immunogen. *J Immunol* 1997;158:816-26.