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Original Research Article

Estimation of Antibody Titre of Hepatitis B Surface Antigen among Medical Students of a Tertiary Care Centre in North Kerala

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Abstract

Background: Hepatitis B virus (HBV) infections at younger age lead to chronic liver damage and hepatocellular carcinoma. Medical students have high risk of exposure to HBV. Vaccination certificate is mandatory for medical course admission as it is vaccine preventable. The immune response wanes gradually over time and antibody level was needed to be maintained by booster dose. Objective of the study was to find out anti-HBs titre and to evaluate the seroprotection in students of Govt. Medical College, Manjeri in North Kerala.

Methods: A cross sectional study was conducted among 375 students of Govt. medical college, Manjeri in north Kerala during 2018-2019. Anti-HBs titre was estimated in serum samples by quantitative ELISA method using direct antibody sandwich enzyme immunoassay kits (Monolisa Anti- HBs PLUS 72566).

Results: Among the 375 participants, 149(39.7%) were completely vaccinated, 90 (24%) had partial immunization and 136 (36.27%) had no vaccination records. Protective titre of $\geq$10 mIU/mL was maintained by 304(81.06%) students. Good seroprotection >100mIU /mL was seen in 234 (62.4%) and 70 (18.7%) individuals were with 10-100mIU/mL. No significant association was seen with age, gender or body mass index (BMI) to seroprotection. Time since last dose of vaccination and number of doses were associated with good immune response ($p < 0.005$).

Conclusions: Anti-HBs wanes over period of time. Partially immunised individual were sensitised to complete the doses. Fully immunized individuals with <100mIU /mL titre were advised to take single booster dose. Subjects who had <10 mIU/mL after the booster dose were asked to repeat three primary doses. Instead of submitting a vaccination certificate, an electronic register is preferred in Medical institutions to record of HBV seroprotection. Immune status against HBV is to be rechecked at the end of course before starting the internship.

Keywords: Hepatitis B Vaccine, Immune Response, Seroprotection, Booster Dose.

Introduction

Hepatitis B virus (HBV) infection is a global public health problem. In 2019, World Health Organization (WHO) estimated that 296 million people were living with chronic hepatitis B infections and had 820 000 deaths due to cirrhosis and hepatocellular carcinoma [1]. India has a prevalence of 2-4% chronic HBV infection with 50 million people in carrier stage [2]. HBV is highly infectious and a viral load of 10 viral copies are estimated to be the minimum 50% infectious dose [3]. The virus can survive on environment upto 7 days [4] Health care workers (HCWs) have four times higher occupational risk but can be prevented by vaccines effectively and safely [3-5]. As beginners to health care system, medical students may not be aware of the risks of exposure or post exposure prophylaxis. Centre for Disease Control and Prevention (CDC) recommended hepatitis B vaccination for health care workers intramuscularly at 0, 1, and 6 months [4,5]. Immunocompetent adults and children who developed vaccine induced antiHBs levels of >10mIU/ml after 1-2 months of complete vaccination with three or more doses are considered seroprotected and deemed vaccine responders [4-6]. Smoking, obesity, chronic liver disease, presence of human leukocyte antigens (HLA)-DR3, DR7 and DQ2 alleles, absence of the HLA-A2 allele and extremes of age are associated with reduced immunogenicity[7]. Post vaccination anti-HBs levels decline gradually over time [4,8-10]. Immunologic memory induced by the HBV vaccine persists even after anti-HBs declined [10,11]. Among immunocompetent vaccine responders natural boosting of anti-HBs can occur from exposure to HBV [4].

In India HBV vaccination was included in childhood immunization programme by 2011-2012 [2]. The participants were born before this period and most of them were belonged to the northern districts of Kerala where the immunization coverage is low [12].

This study was aimed to find out the seroprotection among medical students of Govt. Medical College Manjeri against HBV infection based on anti HBs titre and to advise the need of additional dose of vaccine to maintain adequate immunity.

Methods

A cross sectional study was conducted in the department of Microbiology, between 2018 March-2019 February after ethics committee approval (IEC/GMCM/14/17dt 26/02/2018). All students joined for MBBS course in Govt. Medical College Manjeri, Kerala were selected for the study. Sample size was calculated based on the study from Kerala reported as 89.5% of the successfully vaccinated persons had protective antibody titres [6]. Minimum sample size was calculated using the formula $n= 4pq/d^2$. For a p of 60% based on a pilot study of MBBS students and for an absolute precision of 5%, minimum sample size required was 369.

All students admitted for MBBS course in Govt. Medical College, Manjeri and submitted Hepatitis B vaccination certificate were included for study. Those who were reluctant to provide personal details or consent and undergoing treatment with steroids or immunosuppressant were excluded from study. There were 375 students enrolled for the study.

After getting an informed consent, data was collected through a proforma regarding age, gender, height, weight, body mass index (BMI), and hepatitis B vaccination status, history of jaundice, diabetes, or blood transfusion. Two ml of blood was collected, serum separated and stored in a deep freezer at -20^0C. Quantitative anti-HBs antibody ELISA test was done with serum samples using Monolisa Anti- HBs PLUS, BIORAD 72566 immunoassay kits following manufacturer's instructions. AntiHBs titre ≥10mIU /mL was considered as the standard

for post –vaccination protection against HBV [4-6]. The effectiveness of the vaccination based on anti HBs titre was analysed with demographic data and vaccination details.

Statistical Analysis

Statistical analysis was done using SPSS version 23.The study variables were expressed as frequency and percentage. Chi square test was used to study the association of titre value with important socio demographic and clinical variables. The association was considered as statistically significant when p value is less than or equal to 5%. Titre values were expressed as Mean ± SD with 95% confidence limits. The difference in mean values were tested by t test/ANOVA.

Results

The study was conducted among 375 students including 123 males (32.8%) and 252 females (67.2%). All were belonged to age group 18-28 years and 222 (59.2%) were 20-22 years old. Immune response after vaccination was assessed based on antibody titre. Anti HBs titre of participants were categorised as <10mIU /mL, 10- <100mIU /mL, and >100mIU /Ml [4].

Protective titre of ≥10mIU/ mL was maintained in 304 (81.06%) individuals. Among the sero protected students, 234 (62.4%) had >100mIU /mL with good immune response after vaccination and in 70 (18.7%) had low but protective antibody response with 10-100mIU /mL. 71 (18.9%) students showed poor antibody response below 10mIU /mL. Overall mean titre in the study group was 147.4 (SD 10.0 with 95% CI). Level of immune protection based on the demographic variables, vaccination status and medical factors were analysed. (Table1 and Table 2).

Table 1: Sero protection based on demographic variables, vaccination status and medical factors

Variables		Total N=375 Number (%)	Anti HBs titre			χ^2	p
			<10mIU /Ml (71) Number %)	≥10mIU /mL (304)			
				10-100mIU /mL (70) Number %)	>100mIU /mL (234) Number %)		
Age (years)	17-19	81(21.6)	19(23.5)	16(19.8)	46(56.8)	4.9	0.302
	20-22	222(59.2)	35(15.8)	39(17.6)	148 (66.7)		
	>23	72(19.2)	17(23.6)	15(20.8)	40(55.6)		
Gender	Male	123(32.8)	28(22.8)	26(21.1)	69(56.1)	3.200	0.204
	Female	252(67.2)	43(17.1)	44(17.5)	165(65.5)		
BMI	Under weight (<18.5 kg/m^2)	70(18.7)	12(17.1)	12(17.1)	46(65.7)	1.800	0.767
	Normal (18.5-24.9kg/ m^2)	252(67.2)	48(19.0)	51(20.2)	153(60.7)		
	Overweight/ obese(>25kg / m^2)	53(14.1)	11(20.8)	7(13.2)	35(66.0)		
Years since vaccination	<1 year	18(4.8)	3(16.7)	2(11.1)	13(72.2)	60.2	<0.001
	1 year	170(45.3)	23(13.5)	30(17.6)	117(68.8)		
	2 years	82(21.9)	10(12.2)	11(13.4)	61(74.4)		
	3 years	35(9.3)	3(8.6)	6(17.1)	26(74.3)		
	>3 years	70(18.7)	32(45.7)	21(30.0)	17(24.3)		

Vaccine - No. of doses	One dose	41(10.9)	18(43.9)	15(36.6)	8(19.5)	59.9	<0.001
	Second dose	49(13.1)	8(16.3)	14(28.6)	27(55.1)		
	Third/ fourth dose	149(39.7)	15(10.1)	13(8.7)	121(81.2)		
	Don't know	136(36.3)	30(22.1)	28(20.6))	78(57.4)		
Blood transfusion	Yes	3(0.8)	0	0	3(100.0)	1.8	0 .402
	No	372(99.22)	71(19.1)	70(18.8)	231(62.1)		
H/O Jaundice	Yes	32(8.5)	5(15.6)	7(21.9)	20(62.5)	.395	0 .821
	No	343(91.5)	66(19.2)	63(18.4)	214(62.4)		

Significant association of immune protection was seen to be associated with the time since last dose of vaccination and number of doses.

Table 2: Association of mean antibody titre with demographic variables, vaccination status and medical factors

Variables		Number	Mean±SD	95%CI	t/F	P value
		375	147.4±10.0	108.7-199.8		
Sex	Males	123(32.8%)	141.6±9.0	111.4-179.8	0.247	0.805
	Females	252(67.2%	151.9±10.0	100.2-236.8		
Age (years)	17-19	81(21.6%)	133.3±10.0	77.8-227.9	0.677	0.567
	20-22	222(59.2%)	163.0±9.0	119.5-222.3		
	23-25	68(18.1%)	108.9±12.5	57.5-205.6		
	>25	4(1.1%)	329.3±3.5	72.7-1479.3		
BMI	Underweight (<18.5kg/m^2)	70 (18.7%)	133.2±11.2	72.9-243.1	3.3	0.039
	Normal(18.5–24.9 kg/m^2)	252 (67.2%)	180.3±9.0	134.7-241.2		
	Overweight/ obese (>25kg/m^2)	53 (14.1%)	89.0±10.0	45.6-173.0		
Years since vaccination	<1 year	18(4.8%)	39.4±13.9	10.3-143.4	3.2	0.014
	1year	170(45.3%)	169.7±9.0	119-241.9		
	2 years	82(21.9%)	163.0±10.0	95.5-277.7		
	3years	35(9.3%)	59.3±17.2	21.6-159.8		
	≥3 years	70(18.7%)	199.3±5.7	126.2-314.5		
Vaccination doses	First dose	41(10.9%)	147.4±10.0	71.1-315.1	0.207	0.935
	Second dose	49(13.1%)	120.5±10.0	62.2-242.1		
	Third dose	143(38.1%)	147.4±11.2	98.7-226.5		
	Fourth dose	6(1.6%)	89.0±7.2	15.2-499.0		
	Don't know	136(36.3%)	163.0±8.0	111.5-238.2		
Blood transfusion	Yes	3(0.8%)	297.9±9.0	126.2-701.2	0.509	0.611
	No	372(99.2%)	147.4±10.0	113.7-191.1		
H/o Jaundice	Yes	32(8.5%)	543.6 ±3.5	319.4-924.5	3.2	.001
	No	343(91.5%)	133.3±10.0	102.6-173.0		

Higher mean antibody titre (163.0±9; 95% CI 119.5-222.3) and adequate seroprotection was seen in 187(84.2%) students of 20-22 years. Mean antibody titre 151.9 (SD 10 with 95% CI) of females was higher compared to the males. Protective anti HBs was found in 95/123 males (82.86%) and 209 /252 females (82.9%). Significant association was not seen with antibody titre and age and sex (p>0.005).

Higher mean antibody titre (180.3±9.0) among the normal weight category was found significant (t=3.3; p=0.039). No statistical significance was noticed for the increased protection among the 204/252 (80.9%) of normal weight subjects (p=0.767).

Students were vaccinated at different time period with varied doses. Inadequate antibody titre was more prevalent among 32(45.7%) who were vaccinated beyond 3 years. Antibody titre >100 mIU/mL was seen in 68.8%-74.4% of individuals vaccinated within 1-3 but was reduced to 24.3% when the interval was increased which was found statistically significant (p<0.05).

Sero protection was increased 56.09% to 89.93% on receiving 1-3 doses of vaccination. Immune response increased >100 m IU/mL successively after the first, second and third doses (19.5%, 55.1%, 81.2%). The number of vaccine doses received was significantly associated with protective titre levels (p<0.001) but was not related to mean antibody titre. (p=0.935).

On analysing the medical history, all the three blood recipients had adequate anti HBs. Blood transfusion or jaundice had no association with seroprotection statistically (p>0.05) but jaundice showed significant association to mean antibody titre (543.6 ±3.5; 95% CI 319.4-924.5 t=3.2, p=0.001).

Discussion

Hepatitis B is highly transmissible and could be effectively prevented by vaccination. Anti-HBs is the serological marker to assess immune protection [5]. The Medical students are trainees taking baby steps to health care system, are not aware of the situation. As the HB vaccination certificate was mandatory, some of them received a random dose irrespective of vaccination status or without testing antibody titre.

Ant- HBs antibody profile of 375 students were analysed. 304 (81.06%) of them were immunoprotected which was compared with other studies (Table3).

Table 3: Prevalence of HBV seroprotection in different studies.

Year-Publication & Author	Place of study	Study period	Type of study	Sample size	Study group	Method of testing	Seroprotection ≥10mIU/mL n(%)
2021-Kumar D *et al* [2]	Maharashtra	2018-2019	Cross sectional	122	Immunized children	ELFA*	84(68.86)
2019-Nagashima *et al* [3]	Hiroshima, Japan	2021-2016	---	491	Medical students	CLIA**	474 (96.5)
2021-Bhama *et al* [8]	South Kerala, India	2016	Cross sectional	211	Fully vaccinated HCW	ELISA	188 (89.1)
2014-Sernia *et al* [10]	Rome, Italy	2004-2009	Retrospecti-ve	369	HCWs	-	181 (49.6)
2017-Lakshmanan etal [11]	Tripura, India	2016	Cross sectional	330	All ages	ELISA	287 (86.96)

2020-Regha *et al* [13]	Central Kerala, India	2016	Cross sectional	50	Fully vaccinated HCW	ELFA	48 (96)
2013-Mahawel etal [18]	Dehradun, India	2010-2011	Cross sectional	112	Fully vaccinated HCW	ELISA	91 (81)
2015-Pavani *et al* [21]	Hyderabad, India	2014	Cross sectional	200	HCWs	CLIA	167 (83.5)
Current study	North Kerala,India	2018-2019	Cross sectional	375	Medical students	ELISA	304 (81.06)

* ELFA Enzyme –linked fluorescence assay ** CLIA Chemiluminescence immunoassay

Seroprotection for HBV was found adequate in 304 (81.6%) vaccinated subjects this study which was found lower compared to other studies [3, 8, 11, 13, 14]. Seroprotection was seen to be higher when other study reports were compared [2,7,10]. The lower seroprotection in our study may be attributed to that only 149 (39.7%) students had completed the three primary vaccination doses by records. Immunisation coverage is low in our study area and nearby districts [12] where most of the students were hailing from.

Among the 375 students females were predominant 252 (67.2%). Protective level of anti HBs was possessed by 95 males (77.3%) and 259 (83%) females. There was no statistical significance by gender and sero conversion rate. ($p = 0.204$; $\chi2 = 3.200$) as found in other studies [5,11]. Studies reported that females exhibit greater cell- mediated and antibody mediated immune responses on exposure to antigens, vaccines and infections leading to higher antibody levels [15,16].

All the study participants were belonged to younger age between 18-28 years. Higher immune response was found among 20-22 years group in 187(84.2%) students as they held the majority, 222(59.2%). Antibody titre or mean antibody titre were not statistically associated with age ($P < 0.05$). The inverse relationship between age and anti HBs titre was reported by other studies [5, 10, 11, 13, 17, 18].

Studies found that overweight or obesity (BMR>25 kg/m^2) negatively affects the immune response triggered by HB vaccine [18]. Mean antibody titre (180.3 $\pm$9.0; 95% CI: 45.6-173.0) was found higher in 252 (67.2%) students with normal BMI than the underweight or over weight ones. Statistically significant association was seen between mean titre and BMI ($t=3.3$; $p=0.039$). But significant association was not seen with protective titre of individuals and BMI ($\chi2 = 1.800$; $p = 0.767$) as found in other study reports [15]. One study from China in 2016 remarked that participants with BMI<18.5kg/m2 presented a significantly higher anti HBs than those with BMI>25kg/m [18].

Based on the interval after the last dose of vaccination, the HBs antibody titre was analysed. 265(70.67%) persons developed adequate protection level within 1-3 years which was reduced to 38(54.3%) after 4 years of immunization. Significant negative association was seen with time since previous vaccination and mean antibody titre as well as ($t=3.2$; $F=0.014$) sero protection rate [($\chi2 = 60.2$; $p < 0.005$). Sahana *et al* of Karnataka noticed a fall of mean antibody titre 128.4 ± 46.2 to 102.5 ± 63.4 after 5 years and 93.2 ± 66.1 after 10 years [5]. Mahawal *et al* from Dehradun observed protective antibody levels in 99.9% of subjects one year after vaccination was decreased to 80.96% and 46.16% after 5 years and 10 years of vaccination respectively [19]. According to Kumar *et al,* 59% participants had protective levels of anti-HBs 6 years after childhood immunization and was declined to 13% after

11 years [20] and was supported by other studies [8,10,11,18].

Only 149 (39.7%) participants were completed primary vaccination and 90(24%) was partially immunized. Vaccination status of 136 (36.3%) students were not known due to lack of records of childhood immunization. Many of them submitted the vaccination certificate without checking for antibody titre. Higher prevalence of poor immune response with <10mIU/mL was seen among 18 (43.9%) students who received only a single dose of vaccine. Whereas good antibody response (>100mIU/mL) was seen to be increased successively as 19.5%, 55.1% and 81.2% with one, two and three vaccine doses. Among the completely vaccinated participants, poor immune response (<10mIU/Ml) was noticed in 15 (10.1%) students. Statistically significant association was seen with immunoprotective anti HB titre level and primary vaccination doses (($\chi 2$ =59.9; p< 0.001) but was not found statistically associated to the mean titre. (t=0.207; p=0.935). Declining of immune response with partial immunization was reported in other studies [8,10]. A study from Kerala reported seroprotection in 91.87% of completely vaccinated HCP while it was 80.39% among incompleted vaccines [8]. One Italian study remarked that insufficient protection was more in persons received a single dose of HB vaccine (78.6%) rather than who had two doses (71.4%), three doses (46.4%) [10]. According to the Canada Communicable Disease Report, four dose vaccine schedule did not confer better protection compared to three doses [9].

There was various opinion regarding persistence of protective antibody level over time. Studies reported that maximum anti-HBs titre level occurred within 1- 2 months after the last vaccine dose and 90% of it was reduced further by two years followed by a very slow decline [11,19,20]. Gara N *et al* from Mary land reported that after primary vaccination immunological memory to HBs Ag persists for upto 28 years [21]. Lakshmanan *et al* from India reported that HBV vaccine induced immunologic memory persists for a minimum period of 10 years even though anti-HBs declined [11]. Declining trend was attributed to differences in the genetic and environmental factors, type and dose of the vaccines, age of vaccination, and intervals between vaccine administrations.

There was no history of any comorbidities like diabetes or chronic medication to any of the participants. 32(8%) individuals had history of jaundice in their childhood or adolescent age. 3 (0.8%) students revealed history of blood transfusion following some acute reasons. Statistically significant association was not seen with these factors and anti HBs titre. (P >005).

Studies recommended that revaccination with ≥1dose increase the seroprotection and booster vaccination was required in low or nonresponders [3-6, 7, 8, 11, 14, 17, 18, 21]. Studies reported that persons who had low (1–9 mIU/mL) levels of anti-HBs after the primary vaccination showed better response to revaccination than those do not had measurable anti-HBs levels. According to Jachuk *et al* 9% of nonresponders to primary vaccination showed protective seroconversion after an additional 4th dose [22]. In 2018, a study from Bangkok suggested that 88–94% of young adults with anti-HBs <10mIU/mL developed protective anti-HBs with single booster dose [23]. Vesikari *et al* remarked that immunogenicity of HB vaccine has to be improved to maintain higher antibody concentrations [24]. In a meta-analysis study, Mahmood *et al* found that the risk of poor immunoprotection (Anti-HBs titer ≤10mIU/ml) was lowered by 50% after receiving booster dose [25]. Averhoff F, *et al* found vaccine-induced seroprotection with a single additional dose of HepB vaccine in 47% of initial nonresponders and was increased to 69% after three revaccination doses [26]. In a Thailand study, only 2.1% of

the low responders failed to raise anti-HBs ≥10 mIU/ml after revaccination with 3 doses [27]. A study from Japan suggested to use the same brand of vaccine for non-responders for booster doses. They remarked that changing the type of HB vaccine was associated with polymorphism of human leukocyte antigen (HLA) causing low or non-response to the HB vaccine efficacy [3]. CDC emphasize to check anti HBs titre for all vaccinated HCWs but was not done routinely due to lack of awareness as well as diagnostic facilities [28].

As the antibody titre decline over the period of time a value beyond 100mIU/mL after three doses was considered for good immune protection. The partially immunized students were advised to complete the vaccination whatever may be the titre level. The low responding individuals were advised to take one booster dose and to check the antibody titre after 2 months. Participants whose antibody levels remained <10mIU/mL after complete vaccination and booster dose were advised to revaccinate with three primary doses and to check antibody titre after one month.

Conclusions

The study participants differ in Hepatis B immunization status. The risk of HBV exposure is high for medical students and are needed complete the vaccination with three primary doses. Instead of submitting a vaccination certificate, an electronic register for vaccinations and antibody titre levels is advised to maintain in medical institutions. Rechecking of Anti HBs titre to assess the immune status at the end of course but before starting the internship is advisable.

This study had got some limitations that most of the students had no records of childhood immunization. Many had taken vaccination just before joining the medical institution. So the seroprotection was assessed with the real time antibody titre.

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