



SEROLOGICAL PROFILE OF HEPATITIS B VACCINATED PERSONS WITH SPECIAL REFERENCE TO QUANTITATION OF ANTI-HBs ANTIBODIES TITERS

Dr. Sireesha Chava

Assistant Professor, Department of Microbiology, Kamineni Institute of Medical Sciences, Narketpalli.

ABSTRACT **BACKGROUND:** Hepatitis B Virus infection is a serious global health problem with risk of contracting HBV by health care workers is four- times greater than that of general adult population. High risk groups such as health care workers should be vaccinated and receive a booster vaccination if their anti-HBs Antibodies levels decrease below 10mIU/ml.

OBJECTIVES: My objectives of this study was to evaluate the efficacy of vaccine by determining the antibody titres in various Groups of hepatitis b vaccinated persons.

METHODS: Blood samples from 100 Health Care Workers were tested for HBsAg, anti-HBe, anti-HBc and anti-HBs antibodies by ELISA method.

RESULTS: anti-HBsAg antibodies titers in vaccinated health care workers, 84/90 (93.3%) developed protective levels of antibodies. It was observed that females(97%) have a higher prevalence of anti-HBsAg antibodies than males(85%). According to duration elapsed since vaccination it was observed that higher titers are seen in vaccinated individuals of <5years Duration(97.3%) and amount of titers decreased after 10yrs of vaccination (76.90%).

CONCLUSION: These studies show that complete vaccine administration must be taken and that the ability to respond is influenced by several factors including genetic factors, age, sex etc.

KEYWORDS : Hepatitis B, Vaccination, Health care workers, Anti-HBs titers, Booster dose.

INTRODUCTION

Hepatitis B Virus infection is a serious global health problem, with 2 billion People infected world-wide and 400 million suffering from chronic HBV infection.^[1] It is estimated that between 5,00,000 and 7,00,000 people with chronic HBV infection die of hepatocellular carcinoma or cirrhosis of liver each year.^[2] Annual exposure of health care workers to hepatitis B virus infection was estimated world-wide as 5.9%^[3]. The important parenteral routes of transmission, which may result from accidental inoculation of minute amounts of blood or fluid contaminated with blood during medical, surgical and dental procedures; immunization with inadequately-sterilized syringes and needles, intravenous and percutaneous drug abuse. Perinatal infections lead to a high rate of chronicity, estimated at around 90%, and individuals infected at such an early age exhibit a high degree of immune tolerance and may remain viraemic for decades^[4].

Hepatitis B vaccine, which was developed for preventing HBV infection, contains HBsAg, one of the viral envelop proteins. A course of three vaccine injections is given, with the second injection at least 1 month after the first dose and the third injection given 6 months after the first dose^[5]. Vaccination is not the same as immunization. Just because a person has been vaccinated, it is not necessary that he has been rendered sufficiently immune to the disease. Ideally, one should receive a complete course of hepatitis B vaccine, that is, at least three doses. After that, it is advisable to perform an anti-HBs assay to confirm whether the antibody titer was greater than or equal to 10mIU/mL^[6].

Despite of the type/dose of vaccine, or route of administration, about 5%–15% of all individuals fail to seroconvert to anti-HBs>10mIU/mL with the standard vaccination schedule. Several factors are possible to affect adversely the antibody response to HepB including male gender, advancing age, overweight, smoking, immunosuppressed groups, markers in the human leukocyte antigen (HLA) region of healthy individuals.^[7] Vaccines can show their immune response differently. Responders are those who mount post-vaccination anti- HBs levels of >10 mIU/mL. Non-responders post-vaccine anti- HBs levels remain< 10 mIU/mL even after two months of the last dose of vaccine and test negative for HBsAg and anti-HBc Ab^[8].

MATERIALS AND METHODS

The present study was a type of Cross-sectional study conducted in the Department of Microbiology, J.J.M. Medical College, Davangere.. 5ml of blood was collected from 100 Health care workers during study period from February 2014 to July 2015 by venipuncture under all aseptic precautions. The serum was separated and stored at -20 °C till use. A Standard proforma was documented regarding Bio data including name, age, sex, religion, occupation, residence, marital status and Vaccination History including last date of vaccination,

number of doses of vaccination, history of any exposure to Liver diseases, Jaundice, blood transfusion, sexually transmitted disease and needle prick injury.

INCLUSION CRITERIA :

Healthy persons between age groups of 6-60 years, vaccinated with complete hepatitis B vaccination schedule, duration of vaccination is minimum of 3 months after last dose of vaccination.

EXCLUSION CRITERIA :

Incomplete vaccinated, proved HIV positive, proved Hepatitis C positive, auto immune diseases, Chronic kidney disease and organ transplantation.

To rule out the antibodies developed are solely due to vaccination and not due to infection, sera was tested for HBsAg by HEPACARD, anti-HBe antibodies by “DS-EIA-ANTI-HBe” kit and anti-HBc Ab by “DS- EIA-ANTI-HBc” kit. Non-reactive samples were processed for anti- HBs antibodies titer by The kit “DS-EIS-ANTI-HBsAg” intended for qualitative and quantitative detection of antibodies to hepatitis B surface antigen. Except the hepacard rest of the kits are based on ELISA based methods.

A standard curve which is used for the detection of anti-HBsAg antibodies concentration with the OD values of every Positive control is provided along with kit insert. For the detection of anti-HBsAg antibodies concentration in tested sample mark the average OD value of tested sample on the Y-axis, draw a straight line till the crossing the calibrating curve and from dot crossing drop a perpendicular on X-axis. The dot of the perpendicular crossing with the X-axis will correspond to the anti-HBsAg Antibodies concentration in the tested sample.

RESULTS:

Among the 100 samples collected, all the samples tested were non-reactive for HBsAg by HEPACARD, 5 samples were reactive for anti-HBe antibodies and 5 samples were reactive for anti-HBc antibodies, remaining 90 samples were processed for anti-HBs antibodies quantitative titers. Results were Statistically analysed using IBM SPSS version 20 for Windows

Table 1: Percentage of HCWs protective to vaccination

Susceptible	Frequency	Percent
Yes	6	6.7
No	84	93.3
Total	90	100.0

Anti-HBsAg antibodies titers in vaccinated health care workers,84/90 (93.3%) developed protective levels of Antibodies to HBsAg. Sex wise distribution, it is observed that females (97%) have a higher prevalence of anti-HBsAg antibodies than males (85%).According to age wise, <40 years of age group of HCW's have more protective antibodies(97%) than >40yrs of age group persons(80%).In group wise observation doctors have a more anti-HBsAg antibodies titer compared to lab technicians. According to duration elapsed since vaccination, it is observed that higher titers are seen in vaccinated individuals of <5years Duration(97.3%) And amount of titers decreased after 10yrs of vaccination(76.90%).In booster dose taken individuals, 100% were having antibodies titers >10mIU/ml where as 93% were immune in non-booster individuals, but statistically this was not significant. In diabetic persons, (67%) and in non-diabetic(94%) were immune, but statistically this was not significant. Persons having needle prick injury were having more titers(100%) than without any prick(93%) but statistically this was not significant.The quantity of titers decreases as the duration of vaccination increases but still immune to infection(>10mIU/ml).

TABLE 2: Percentage of persons in three different categories of anti-HBsAb titers

anti-HBsAb titers			
Categories	Frequency	Percent	
Ab titers < 10mIU/mL	< 10	6	6.7
Ab titers > 10mIU/mL	10 – 100	34	37.8
	> 100	50	55.6
Total	90	100.0	

TABLE 3:Age wise distribution of anti-HBsAb titer in HCWs

Age distribution	No. of cases	Ab titers < 10mIU/mL		Ab titers > 10mIU/mL		Chi square / Fisher's exact Test
		No. of cases	%	No. of cases	%	
<40yrs	70	2	3	68	97	7.35,
>40yrs	20	4	20	16	80	P<0.009
Total	90	6	9.7	84	90.3	

TABLE 4: Sex wise distribution of anti-HBsAb in HCWs

Sex distribution	No. of cases	Ab titers < 10mIU/mL		Ab titers > 10mIU/mL		Chi square / Fisher's exact Test
		No. of Cases	%	No. of cases	%	
Male	27	4	15	23	85	4.16,
Female	63	2	3	61	97	P<0.04
Total	90	6	7	84	93	

Table 5: Percentage of vaccines protective based on time elapsed since last dose of vaccination.

Vaccination (In years)	No. of cases	Ab titers < 10mIU/mL		Ab titers > 10mIU/mL		Chi square/ Fisher's exact Test
		No. of cases	%	No. of cases	%	
< 5	37	1	2.7	36	97.30	14.2,
5 - 10	40	2	5.0	38	95.00	P<0.007
> 10	13	3	23.1	10	76.90	
Total	90	6	6.66	84	93.30	

TABLE 6:Distribution of anti-HBsAb titers in percentage based on time elapsed since vaccination

Vaccination (In years)	Total No. of cases	anti-HBsAb titers			% of cases with anti-HBsAb > 100	% of cases with anti-HBsAb < 10	% of cases with anti-HBsAb 10-100
		< 10 mIU/mL	10-100 mIU/mL	>100 mIU/mL			
< 5	37	1	9	27	73.00%	2.70%	24.32%
5 – 10	40	2	18	20	50.00%	5.00%	45.00%
> 10	13	3	7	3	23.10%	23.00%	53.84%
Total	90	6	34	50	55.50%	6.66%	37.77%

TABLE 7: Percentage of persons protective who had taken booster dose

Booster	No. of cases	Ab titers < 10mIU/mL		Ab titers > 10mIU/mL		Chi square / Fisher's exact Test
		No. of cases	%	No. of cases	%	
Yes	7	0	0	7	100	0.54, NS
No	83	6	7	77	93	
Total	90	6	7	84	93	

DISCUSSION:

In the present study, the efficacy of vaccine is 93.3%.The number of vaccinated persons protected from vaccination was 93.3% and 6.7% of vaccinated persons are not protective by vaccination, and required further vaccination. which are coinciding with Study by Jennifer Perera et al., in which 92.1% were protective where as 7.9% were non-protective.^[9] and study by Abdul Ahad et al., 88.67% were protective and 11.33% were non-protective.^[10]

Present study was not coinciding with Study by Bidhan chakraborty 100% were protective and 0% were non-protective.^[5]

TABLE 8: Distribution of anti-HBsAg antibody titers in three categories in HCWs in various studies

Various studies	<10mIU/mL	10-100mIU/mL	>100mIU/mL
Seyed Moayed Alvian ^[11]	5.9%	16.9%	77.3%
Marinho et al ^[12]	5.2%	27.5%	67.3%
Jennifer Perera et al ^[13]	9.5%	36.5%	54%
Present study	6.7%	37.8%	55.6%

TABLE 9: Age group wise distribution of anti-HBsAb titers in various studies

Janbakhsh et al ^[14]	<40yrs	76.1%
	>40yrs	58%
Seyed-Moayed Alvian et al ^[11]	<45 yrs	82.2%
	>45yrs	63.6%
	Present study	
	<40yrs	97%
	>40yrs	80%

In the present study, the gender wise distribution females were 97% immune to infection as compared to males in whom 85% were immune to infection(p<0.04). Study by Majda Qureshi et al., 57% of females responded to vaccination where as 43%of males responded.^[15] Study by Mohd.Abdul et al., 85.88% of males responded where as 92.3% of females responded^[7]. Ferraz et al.,found a higher titer of antibody in females than males^[16] these studies are correlating with present study.

In the present study,the profession wise distribution of titers, dental professionals were 100% immune to infection, in medical professionals 96% were immune to infection and lab technicians 75% were immune to infection.(p<0.004).In a study by Sabina Serniac et al.,the level of antibody titers are not significantly different by type of course of study^[17].The above results do not coincide with the present results. The results might be varied in my study assording to knowledge of vaccination in different staff.

In the present study, according to category of time elapsed since vaccination, in<5yrs 97.3% persons were immune, in 5-10yrs since vaccinated 95% were immune and in >10yrs of time elapsed since vaccination 76.90% were immune to infection.(p<0.007) In a study conducted by A.Eldesoky et al,the percentage of people immune in <5yrs was 82.6%,within 5-10yrs it was 42.8% and >10yrs it was 33.3%^[19] and study by Mehdi et al, 88.1% was protective <5yrs,88.9% were protective in 5-10yrs and 60.9% were protective >10yrs^[18] both the above study results were coinciding with present study results.

In the present study, according to category of booster dose, persons who had taken booster doses were having 100% >10mIU/ml titers , where as in persons who had not taken booster dose it was 93%. (p=0.54). Surgedr CNChandhari et al,there was no difference in protection in booster and non- booster groups. In this study, adequate titers were 100% in people who have taken booster and 93% in people who have not taken booster, these results are coinciding with present results^[20].

In the present study, the number of tested diabetic persons were 3 of 90 persons. Seroprotection rate was 67% in diabetic persons, while it was 94% in non-diabetic persons but statistically this was not significant ($p=0.06$). In the study by Bouter et al., it was 75% seroprotection in diabetic persons and 96.9% in non-diabetic persons^[21].

Considering needle prick injuries, professionals who sustained needle prick were 100% immune to infection compared to persons who did not have needle prick injury with 92%, but statistically this was not significant ($p=0.41$). In study by Nafiesh Momeni et al., no significance was found between levels of antibody titers and number of needle prick injuries.^[22]

CONCLUSION:

According to my study hepatitis B vaccination is the best preventive way for healthcare workers from getting hepatitis B infection. But the response to the vaccination differs by sex, age, genetic predisposition other health conditions of healthcare workers and also time elapsed since last dose of vaccination. So every one should get their titers checked after vaccination and also before taking booster doses. If titers are not adequate then they should be vaccinated again.

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