Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.17, 2013

Immune Response to Hepatitis B Virus Vaccine

AYAM MOHAMMED SALIH ALI(Corresponding author)
Department of Biology, College of Basic education, University of Babylon.
E-mail of the corresponding author: ms_ay20@yahoo.com

Abstract

Hepatitis B virus (HBV) infection is a major public health problem and its outcome depends on the kinetics of the virus host interaction and in particular on the strength of the immune response.this study was done to illustrate some immunological in vaccinees and effectiveness of HBV vaccine in vaccinees groups . This study was conducted from September , 2011 to October, 2012 . ELISA assay was used for detection of hepatitis B virus surface antigen as well as IgM- anti hepatitis B-core antigen . This study was also include measurement of anti- HBsAg level to detect the immune response in vaccinees . It was found that significant differences in the antibodies levels according to post vaccination periods and the highest antibodies level in male and female was reached after 90 days post 3rd dose of vaccination , it was 124.85 mIU/ml and 155.94 mIU/ml in male and female respectively. There was also significant differences in (IL-2 it was found that the highest level of IL-2 was reached 27.21 pg/ml and 33.94 pg/ml 90 days post 3rd dose of vaccination in male and female respectively.

Keyword: Hepatitis B vaccinees, anti-HBsAg, HBsAg, anti-HBcAg, IL-2

1.Introduction

The term (Hepatitis) is applied to a broad category of clinical, pathologic conditions, charcterized histologically by hepatocellular necrosis and inflammatory cell infiltration of the liver (Pondei & Ibrahim 2013). Hepatitis results from hepatocyte damage produced by the action of varing form of chemical agents, including, drugs, toxins, alchohol or some pathogens such as, viruses and others (Tannapfel *etal*. 2011). However, virus still represents the most common and important causative agent of hepatitis caused by at least five different viruses which are ecologically, immunologically, and epidemiologically distinct , Viral hepatitis types are: A (formerly called infectious hepatitis), B (serum hepatitis), C (formerly called post-transfusion non-A non-B hepatitis), D (Delta hepatitis), E (Enterically transmitted). F and G, cryptogenic (caused by a virus as yet unidentified), (Das &Maini 2010) .

HBV is structurally complex that it belongs to a group of animal viruses known as Hepadnaviridae. It is 42 nm in diameter and consists of two primary components: a DNA, viral core and an outer protein coat (Locarnini & Zoulim 2010). The viral core represents the infectious part of the virus and the outer coat carries the major antigenic determinant of the virus, the HBsAg. The viral core rests within the nucleocapsid of the virus. It is approximately 27 nm in diameter and contains partially double stranded DNA , DNA polymerase , a core antigen (HBcAg) , and an e antigen ((Pondei & Ibrahim 2013).

The outer coat of the virus consists of varied combinations of proteins and includes the important, yet non-infectious, surface antigen (HBsAg). Most of the excess, non-infectious protein (HBsAg) is released into the blood stream in the form of 22 nm spherical particles and filaments of the same diameter with variable length up to 200 nm(Tiollais There are four major subtypes of HBsAg (adw , adr , ayw and ayr) having a group specific determinants (a), shared by all HBsAg and type specific sub-determinants d, y, w, and r and several further categories of subtypes, named adw2 , adw4 , ayw3 , ayw4 (Kondo *etal*. 2013)

Vaccination was the most effective strategy for control and eradication of HBV infection and it has been one of the great technical development contributing to the freedom from life-threatening infectious disease ,

Hepatitis B vaccine have been commercially avaliable since 1982, it consists of a small protein from the surface of the hepatitis B virus called Hepatitis B surface antigen (HBsAg). The first HB vaccine was manufactured by the purification and inactivation of HBs Ag obtained from the plasma of chronic hepatitis B virus carriers called Plasma Derived Vaccine (Faas *etal*. 2011) .

HBV vaccine should be administred in deltoid muscle of adults, adolescents and children or in the anterlateral thigh muscle of neonates, infants and chlidren in the second years of life in three doses at intervals of (0 , 1 and 6 months) ,All Licensed formulation for both vaccine produce high rate (95%) of protective antibody (Anti-HBs Ab > 10 mIU/ml) when complete vaccine doses (Ryckman *etal*. 2010). The availability of high safe and effective hepatitis B vaccines now makes possible the establishment of programs aimed at eventual elimination

of HBV as a disease in man and the prevention of the first human cancer by a program of immunization. World wide strategies for hepatitis B prevention will differ from area to area according to the epidemiology of HBV infection(Faas *etal*. 2011) .

People with risk factors for HBV infection should be vaccinated by using the age appropriate vaccine dose and schedule ,these include people at accupation risk of infection through exposure to blood or blood products they including Health Care Workers (Laboratory technicians, Dentistry, Surgeon, Nursing and other), medical students, house hold contact to HBV carriers ,users of illicit drugs, Hemodialysis patients, Insulin Dependent Diabetes Mellitus IDDM patients,infant born to HBsAg positive mothers, clients staff of instutions for the developmentally disable, recipients of certaine blood products such as clotting factors and international travelers especially in area with high or intermediate of HBV infection (Alkandari *etal*. 2013).

2.Samples Collection and Processing

Total of (20) unvaccinated healthy individuals (10) female and (10) male at age group between (20-21) years were tested for HBsAg , anti-HBcAg , anti-HBsAg and estimation of certain cytokine levels (IL-2) before and after vaccination for estimation effectiveness of HBV vaccine in vaccines group . Vaccination by using **(EUVAX-B) (LG, Korea),** . Determination of anti-HBs antibodies after complete the vaccination program (If anti-HBsAb < 10 mIU/ml was considered as non responders) While Anti- HBsAb ≥ 10 mIU/ml was considered as responders.
Twenty control group un vaccinated. Blood sample of 5ml were obtained from each subject by using sterile syringes. Each blood sample was collected in sterile plan tube , labeled then all samples were incubated at room temperature till clotted. The serum was separated from collected blood by centrifugation in Kokusan (Japan) , centrifuge at 3000 r.p.m for 10 min. and transferred into labeled tube (3tube of each subject) each tube contain 100mμ . Each sample was tested for HBsAg detection (ACON company, USA) ,anti- HBcAg (Biokit company, Spain) , anti-HBsAg (Biokit company, Spain) as well as IL-2 test (eBioscience company, USA). only cases of negative result for all HBV markers were vaccinated. The study include twenty healthy subject a Control group.

Euvax B is given only by intramuscular rout .

- In pediatric (children aged up 5 to 15 years of age) the dose was 0.5 ml containing 10 *M*g of HBsAg.
- In adult (from 16 years and up old the dose was 1.0 ml containing 20 *M*g of HBs Ag). While in hemodialysis patients the vaccine dose was double containing 40 Mg of HBs Ag.

3.Statistical Analysis

Data were analyzed statistically using complete randomized design(CRD), LSD and X2 test (Naizi 2004).

4.Results and Discussion

4.1 Virological antigen test.

The results of virological antigen diagnosis for subjects before vaccination shows negative result for each HBsAg,anti- HBsAg and anti-HBcAg. Table (1) show diagnosis of virological antigen for subjects before vaccination.

Table 1. diagnosis of each HBsAg, anti- HBsAg and anti-HBcAg in sera of sera of vaccinees before HBVvaccination

sex	samples	HBsAg Negative(-)	Anti- HBcAg Negative(-)	Anti-HBsAg Negative(-)
male	10	10	10	10
Female	10	10	10	10

Precedent studies show that necessary detection of anti- HBcAg for subjects before vaccination for confirmation that immune against hepatitis B virus cause by vaccine not infection , also anti-HBcAg test using to detection recent and old infection because anti-HBcAg still for life (Hall 2010). Antibodies of hepatitis B surface antigen appear during infection or vaccination (Passos *et al*. 2011).

Table (2) show value of titer for IL-2 in vaccinees before vaccination for use this value for comparative with IL-2 result after vaccination.

Table 2. Constration of IL-2 cytokine in sera of sera of vaccinees before HBV vaccination

Cytokine	Concentration (pg/ml) M±SD	
	female	Male
IL-2	2.61± 1.38	1.32± 0.15

Test of IL-2 cytokine for subjects before vaccination show don't increased in concentration of IL-2 . Titer of IL-2 cytokine different before and after vaccination , level of IL-2 is lower in sera of subjects before vaccination than these after vaccination (Jincheng *etal*. 2011).

4.2 Titer of Anti-HBsAg of vaccinated after vaccination

Detection of anti- HBsAg by using ELISA assay show significant differences in the antibodies level according to post vaccination period and the highest antibodies level in male was reached after 90 days post 3rd dose of vaccination , it was 124.85mIU/ ml . female shows highest antibodies level after 90 days post 3rd dose of vaccination , it was 155.94 mIU/ ml. While the period 14, 21 and 28 day don't show any immune response in male, because titer of antibodies was reached to 2.70 mIU/ ml, 6.70 mIU/ ml and 8.41mIU/ ml respectively whereas in female the period 14 and 21 day don't show any immune response because titer of antibodies was reached to 2.83 mIU/ ml and 7.62mIU/ ml respectively . Result shows significant differences comparative with unvaccinated control group ($P< 0.05$). table (3).

Table 3. concentration of anti-HBs in sera of vaccines after HBV vaccination.

sex	**dose**	**subjects**	**Level of anti-HBs (mIU/ ml) M±SD**				
			Period (days) post vaccination				
			14day	**21day**	**28day**	**60day**	**90day**
male	First	control	0	0	0	0	0
		patient	2.70± 1.39	6.70± 4.75	9.29± 8.41	17.19±11.30	24.23±14.14
	Second	control	0	0	0	0	0
		patient	26.70± 15.59	29.06±17.17	37.62±24.89	50.09±34.08	58.20±40.50
	Third	control	0	0	0	0	0
		patient	68.72± 49.58	79.82± 57.31	86.40± 58.90	100.28± 56.34	124.85± 55.69
female	First	control	0	0	0	0	0
		patient	2.83± 1.93	7.62± 2.30	10.46 ± 2.98	10.70± 3.83	11.62±4.17
	Second	control	0	0	0	0	0
		patient	13.32±7.45	18.52±13.90	25.90±19.02	41.03±31.88	57.94± 39±40
	Third	control	0	0	0	0	0
		patient	81.74±47.66	88.12± 53.01	90.04± 53.36	130.65±54.25	155.94± 59.25
LSD(0.05)= 18.261							

***** Significant differences ($p<0.05$).

Efficacy of vaccination is determined by assessment of the level of antibodies to HBsAg (Faas *etal*. 2011). A serum level of at least 10mIU/ ml of anti-HBs antibodies reached after vaccination has been proposed to be the lowest limit for protection (Central for Disease Control and Prevention 2006). However , a small group of vaccinees produce no (<10mIU/ ml) or suboptimal concentration (10-100mIU/ ml) of antibodies which are known as non-responders and low – responders (Ertem *etal*. 2010). The reason for non- and hypo – responsiveness are unclear yat and may involve multiple factors including dose and procedures of vaccination , recipient's age , gene background , immune response (Jincheng *etal*. 2011).

The study also include measurement of IL-2 cytokine by using ELISA assay. Results shows elevation level of IL-2 in sera of vaccination subjects .The highest level of IL-2 in male occur after 90 day post each dose it was reached to 11.58 pg/ml, 21.12 pg/ml and 27.21pg/ml respectively . The highest level of IL-2 in female occur after 90 day post each dose it was reached to 13.82 pg/ml, 28.88 pg/ml and 33.94pg/ml respectively . Result shows significant differences comparative with unvaccinated control group ($P< 0.05$). table (4).

Table 4. concentration of IL-2 (Pg/ml) in sera of vaccines after HBV vaccination

sex	**dose**	**subjects**	**Level of IL -2 (pg/ml) M±SD**					
			Period (days) post vaccination					
			7day	**14day**	**21day**	**28day**	**60day**	**90day**
male	First	control	1.37±0.12	1.46±0.23	1.39±0.30	1.56±0.25	1.62±0.26	1.50±0.26
		patient	6.38±1.07	8.05±2.63	8.62±3.09	10.42±1.04	11.25±3.12	11.58±1.45
	Second	control	1.72±0.15	1.56±0.11	1.56±0.49	2.13±0.25	2.14±0.68	1.59±0.34
		patient	13.04±9.30	15.01±10.50	17.01±24.16	17.80±4.99	20.07±1.57	21.12±8.41
	Third	control	1.86±0.36	1.87±0.87	1.99±0.53	2.01±0.41	2.16±0.81	1.85±0.72
		patient	21.53±20.57	23.01±1.12	23.08±0.78	23.83±14.56	25.12±5.56	27.21±25.37
female	First	control	1.65±0.32	1.52±0.23	1.52±0.25	1.51±0.19	1.34±0.26	1.54±0.25
		patient	4.94±3.33	7.38±1.76	10.34±12.57	10.98±1.49	11.29±0.93	13.82±0.97
	Second	control	1.78±0.51	1.87±0.78	1.83±0.54	1.89±0.52	1.75±0.64	1.94±0.49
		patient	18.37±16.39	18.27±1.42	20.37±1.59	21.39±9.77	25.69±15.36	28.88±2.44
	Third	control	1.98±0.46	1.97±0.44	1.82±0.49	1.87±0.51	1.85±0.67	2.10±0.53
		patient	28.95±0.75	27.39±10.23	30.08±9.61	30.29±14.71	32.22±13.78	33.94±8.33
LSD(0.05)= 7.305								

* Significant differences ($p<0.05$)

Cytokines act as a key role in the regulation of the immune response to hepatitis B vaccine. The present study analyzed the serum level of IL-2 as indicator of immune response to vaccination in different group. T–helper cells (Th) can be functionally distinguished based on the profile of cytokine production. Th_1 cells induce cell mediated immune response by secreting cytokines such as IL-2. On the other hand, Th_2–cells secrete some other cytokines such as IL-4, IL-5, IL-10 and IL-13 seems to be essential for antibody production against HBV vaccine (Dowlati *etal.* 2010). Humoral and cellular immune response against hepatitis B virus antigen are responsible for clearance of the virus and destruction of infected cells leading to possible curing of infected hepatocytes (FitzSimon *etal*. 2013) . Vaccination with HBsAg induces a protective immunity through Th cell dependent on anti HBs antibody production usually subpopulation of T–cells are probably acting as a central role in response to HB vaccine.(Passos *etal*. 2011).

Conclusion

- Anti- HBsAg titer in sera of female who received HBV vaccine is higher titer than male .
- IL-2 titer in sera of vaccination subjects is higher titer than control group

- Immune response in female more than male

References

Alexandrakis, M.G, Passam, F.H, Pappa, C.A., Damilakis, J., Tsirakis, G., Kandidaki, E., Passam, A.M, Stathopoulos, E.N & Kyriakou, D.S. (2005), "Serum evaluation of angiogenic cytokine basic fibroblast growth factor, hepatocyte growth factor and TNF-alpha in patients with myelodysplastic syndromes: correlation with bone marrow microvascular density", *Int. J. Immunopathol. Pharmacol18,287-295.*

Billerbeck, E., Bottler, T.& Thimme, R. (2007), "Regulatory T cells in viral hepatitis", *World J. Castroenterol 13(36), 4858-4864.*

Centers for Disease Control & Prevention. (2006), "A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults", *MMWR 55(16),1-33*.

Chang, J. & Lewin, S. (2007), "Immunopathogenesis of hepatitis B virus infection", *Immunol. Cell Biol.J. 85(1),16-23.*

Chisari, F. V., Isogawa, M. & Wieland, S. F. (2010), "Pathogenesis of hepatitis B virus infection" Pathol. Biol(Paris) 58(4),258-266.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K. & Lanctôt, K.L. (2010), " A meta-analysis of cytokines in major depression" Biol Psychiatry. 67 (5), 446-457.

Ertem, D., Gonen, I., Tanidir, C., Ugras, M., Yildiz, A., Pehlivanoglu, E., Eksioglu- Dermiralp, E. (2010), "The response to hepatitis B vaccine : does it differ in celiac disease". Eur. J. Gastroenterol. Hepatol. J 22(7), 787-793.

Fass, F., Dooern, V., Litjens, N. (2011), "Hepatitis B vaccination: effects of diminishing HBV immunity, nonresponse and a review of the vaccination protocol in the Netherlands", Erasmus J. Med. 2(1),34-37.

Hall A J (2010). Boosters for hepatitis B vaccination? Need for an evidence-based policy. Hepatol. J. 51(5):1485-1486.

Jafar zadeh, A. & Shokri, F. (2003), "Association between HLA antigens and lack of antibody response to recombinant hepatitis B vaccine in healthy Iranian neonates" Scand. J. Immunol. 54, 45-48.

Jincheng, L. I., Deming, T. A. N., Hongbo, L. I.U., Kecheng, L.I. (2011), "CD4 CD25 FoxP3 T regulatory cells in subjects responsive or unresponsive to hepatitis B vaccination", *Cent South* Univ.J. Med. Sci. 36(11):1046-1051.

Kondo, y., Ninomiya, M., Kakazu, E. & Kimura, O. (2013), "Hepatitis B surface antigen could contribute to immunopathogenesis of hepatitis b virus infection" . ISRN Gastraenterology .

Kramvis, A., Kew, M. & François, G. (2005), "Hepatitis B virus genotypes",Vaccine. 23 (19): 2409–2423.

Locarnini, S. & Zoulim, F. (2010)," Molecular genetics of HBV infection". Antivir. Ther. 15(3), 3-14.

Tannapfel, A., Denk, H., Dienes, H. P., Langner, C., Schirmacher, P., Trauner, M. & Flott-Rahmel, B. (2011), "Histopathological diagnosis of non-alcoholic and alcoholic fatty liver Disease", Virchows Arch. 458(5), 511-523.

Musso, T., Cappello, P., Stornello S, Ravarino D, Caorsi C, Otero K., Novelli, F., Badolato, R., Giovarelli, M. (2005), " IL-10 enhances CCL2 release and chemotaxis induced by CCL16 in human monocytes" Int. J. Immunopathol. Pharmacol. 18, 339-349.

Niazi, A. (2004), "Statistical analysis in medical research", 2nd. Ed. College oN On. Antivir. Ther. 15(3), 3-14.

Niedre-Otomere, B., Bogdanova, A., Skrastina, D., Zaiakina, A., Bru vere, R., Ose, V., Gerlich, W. H., Garoff , H., Pumpens, P., Glebe, D. & Kozlovska (2012), " Recombinant Semliki Forest virus vectors encoding hepatitis B virus small surface and pre-S1 antigens induce broadly reactive neutralizing antibodies", J.Viral Hepat. 19(9), 664-673.

Passos, A. M., Treitinger, A. , Spada, C. (2011), "Hepatitis B immunity and vaccination coverage among young adult males in the Air force in south Brazil", Vaccine J. 29, 9284-9288.

Poland, G. A., Jacobson, R. M. (2004), " Prevention of hepatitis B with the hepatitis B vaccine", *N.* Engl. J. Med. 351(27) , 2832-2838

Ryckman, K. K., Fielding, K., Hill, A. V., Mendy, M., Rayco-Solon, P. & Sirugo, G. (2010), "Host genetic factors and vaccine-induced immunity to HBV infection: hapiotype analysis", PLoS ONE. 5(8), 12273.