

Comparative Evaluation of Four Hepatitis B vaccines available in Pakistan: Reactogenicity and Immunogenicity

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Abstract

Aim: Main objective of this study was to evaluate the immunogenicity of hepatitis B vaccines commonly available in the Pakistan's market. For this purpose we compared immunogenicity and reactogenicity of four recombinant hepatitis B vaccines in apparently healthy young female volunteers in Karachi.

Introduction: Today most of the world's people recognize the importance of vaccination and more than 80% of the world children are now immunized against diseases covered by EPI (expanded program on immunization). The Hepatitis B vaccines have been available since 1982 and more than one billion doses have been used. Approximately 100 countries, consistent with World Health Organization policy, have added HB vaccination to their routine childhood immunization programs. Infect many developing countries have scored astonishing success in controlling communicable diseases through mass vaccination and environmental sanitation.

Materials and Methods: A total of 243 apparently young healthy female students of two Universities of the city were included in this study performed during Jan 2003 to Jan 2006, after receiving written informed consent. Four recombinant yeast derived HB vaccine were used as test regimens i.e. Euvax-B (LG Chemicals Ltd., Korea), Heptis-B (Boryang, Korea), Amvax-B (Amson, Pakistan) and Engerix-B (GS & K, Belgium). Participants were injected with the vaccine of their own choice. Information brochures of the four vaccines were distributed among participants to help them make a choice. anti-HBV antibody titres were recorded using EILSA (IMX-ELISA, Abbott).

Results: A total of 243 HBV and HCV negative individuals came forward with the interest for immunization with the Hepatitis B vaccine of their own choice. Out of total 729 doses administered to 243 individuals during this study (Jan'2003 – Jan'2006)....., 195 were of Engerix-B, 420 were Heptis-B, 75 were Amvax-B, and 39 doses were of Euvax-B. Among these four candidate vaccines Engerix-B came up with the least adverse effects, Euvax-B and Heptis-B showed moderate level of side effects, while Amvax-B showed maximum level of side effects. Although, none of these vaccines showed very severe type of adverse effects like demyelination or central nervous system disorders during last 05 years period, except soreness, indurations, swelling, redness, mild pain, granuloma formation, and mild fever at the time of injection or just after injecting the vaccine, which was recovered within couple of hours.

Conclusion: Overall serum protection rate achieved in case of Engerix-B was 95.9%, in case of Euvax-B, it was 95.2%, in case of Heptis-B was 95.0% , and in case of Amvax-B it was 95.1%, which fulfils the WHO requirements for a hepatitis B vaccine (i.e. seroprotection rate of > 95%), P values observed were lesser than 0.05 indicating significance of the vaccines and good safety profile in subjects.

Key Words: Sero protection, Mass Immunization, HBV, Reactogenicity, Immunogenicity

Hepatitis B is one of the world's major health problems ¹. By recent estimates, worldwide more than 2 billion people have been infected with hepatitis B virus (HBV) globally and more than 350 million have chronic (long term) liver infections². The infection is supposed to be causally related to 1 to 2 million deaths per year worldwide ³. Hepatitis B is a blood borne infection that is transmitted 1) by an infected mother to the newborn, 2) by contact with infected blood through unsafe injection, transfusion, open wounds, and sharing toothbrush or razors, and 3) by unprotected sex. Approximately 90% of newborns infected with HBV develop chronic infection, whereas 30-50% of children under age 5 years, 10% of adolescents aged 15 years, and 2-5% of older individuals develop chronic infection ^{4,5}.

In Pakistan, it is Hepatitis (B & C) not Human immuno deficiency virus (HIV) that is the most common serious viral infection. Number of hepatitis B carriers in Pakistan is estimated at around seven million ⁴ that is about 5% of the world wide 350 million carriers of hepatitis B ⁵. Unlike HIV,

there was no large-scale national awareness campaign to educate the public and healthcare professionals in Pakistan about these infections before 2006, but now a comprehensive national strategy that will lead to the elimination and control of hepatitis B is becoming a top public health priority in Pakistan after inclusion of HBV immunization in government's expanded Program for Immunization (EPI). The World Health Organization (WHO) Assembly endorsed the recommendation of its Global Advisory Group that all countries should implement a hepatitis B immunization program ⁶. The threat of HBV to the health of the nation is frequently under-recognized by epidemiologists, policy makers and the public because unlike the influenza virus, it is often not the acute infection that makes people sick, but the consequences of chronic HBV infection that occurs after 20-30 years. Fortunately, hepatitis B is a vaccine preventable disease, global eradication is therefore possible if everyone worldwide receives the HBV vaccine before they become infected. Despite advances in antiviral therapy, only a minority of patients with chronic hepatitis B will have a sustained response. Thus, primary prevention by vaccination to

increase herd immunity remains the main thrust in the control of HBV infection.

The development of hepatitis B vaccine is considered to be one of the major achievements of modern medicine. Three different classes of hepatitis B vaccine are available based upon how they are derived (from plasma, yeast, or mammalian cells). The first generation HBV vaccine was prepared by concentrating and purifying plasma from Hepatitis B surface antigen (HbsAg) carriers to produce 22 nm sub viral particles, which contain HbsAg alone. Derivation from plasma has left lingering concerns regarding the potential to transmit blood-borne infections, although this vaccine has excellent efficacy and safety ⁷. Yeast-derived recombinant HBV vaccines were first introduced in the mid 1980s. They are produced by cloning of the HBV-S gene in yeast cells. These vaccines contain non-glycosylated HBV small S protein as the envelope antigen which must be released from the yeast during the manufacturing process ⁸. These vaccines do not contain antigens of the pre-S regions. The third class of HBV vaccine is the mammalian cell-derived recombinant vaccine. Three vaccines of this class have been developed. In addition to the S antigen, one of these contain antigen from the pre-S2 region while the other two contain antigens from both the pre-S1 and pre-S2 regions ⁹

The efficacy of universal immunization has been shown in different countries, with striking reductions of the prevalence of HBV carriage in children, most importantly; the HBV vaccine can be considered the first successful anti-cancer vaccine, as 20 years of mass vaccination has clearly reduced the incidence of hepatocellular carcinoma in children, at least in Taiwan ¹⁰. Currently available hepatitis B vaccine in Pakistan's market are genetically engineered DNA recombinant vaccines and the recommended series of three intramuscular doses of hepatitis B vaccines induces a protective antibody response (anti-HBs > =10 milli-international units {mIU/ml}) in > 90% of healthy adults and in >95% of infants, children and adolescents ^{7, 11}.

A vaccine consists of many parts, only one of which is the antigen by which it is known. Other components of the presentation may include, for instance, an adjuvant, a preservative or other ingredient. There may be components not stated on the information sheet that are classified as proprietary and therefore the manufacturers are not obliged to declare them. Thus, the effect the vaccine has, on an individual may be influenced in various ways by each and all of these components. Preservatives are just one of a number of additives to vaccines that are carefully regulated and which come under special scrutiny from time to time ¹². Several case reports raised concerns that hepatitis B (HB) immunization might be linked to new cases or reactivation of multiple sclerosis, could shift the immune system toward an auto-immune direction, or may cause central nervous system (CNS) demyelinating diseases etc ^{13, 14, 15, 16}. The present study sought to compare the safety of four hepatitis B vaccine regimens available in Pakistan's market,

in apparently healthy young females, and to determine the sero-response (i.e. reactogenicity and immunogenicity) to these vaccine in the same group of volunteers.

MATERIALS AND METHODS:

Study Duration: Jan 2003 to Jan 2006

Study Design: Prevention, Open Label, Dose Comparison, Parallel Assignment, Safety/Efficacy Study.

Subject: A total of 243 apparently young healthy female students of two Universities of the city were included in this study.

Informed Consent: Prior to immunization, all volunteers were requested to give written informed consent to participate in this study. The volunteers were also advised that they are free to withdraw from the study at any time without any obligation to disclose her reason (s) for so doing.

Criteria For Inclusion In The Study: All volunteers after submitting their signed consents were subjected to selection criteria on the basis of health checkups by a medical doctor to record the various factors including:

- a) Age: 18 – 30 years, b) History of Jaundice, blood transfusion, exposure to syringe, surgical and dental,
- c) Weight: > 45 Kg, d) Body Temperature: 96 – 98°F, e) Hemoglobin: > 10 g/ dl, f) Blood Pressure: Systolic 100 – 180 mm of Hg, Diastolic: 60 – 100 mm of Hg, g) Pulse rate: > 65/min.

After qualifying for inclusion in this study, volunteers were asked to give 10 cc of blood sample for different hematological (CP i.e. complete blood picture and Hb% i.e. hemoglobin percentage by Sysmex blood analyzer & ESR i.e. erythrocyte sedimentation rate by Westergren method), and Biochemical analysis (Direct Bilirubin, Indirect Bilirubin, ALT, AST and, Alkaline phosphatase by MicroLab- Merck chemistry analyzer), this data was used to keep the record of health status of participants and its comparison with adverse effects if appeared. Screening for HBs antigen, anti HBs antibodies and HBc IgM antibodies by Immunochromatography (ICT, Australia and Abbott, USA) and confirmation by enzyme linked immunosorbent assay (IMX ELISA - Abbott, USA) was also done before first dose of immunization.

Test Vaccines: Four recombinant yeast derived HB vaccine were used as test regimens i.e. Euvax-B (LG Chemicals Ltd., Korea), Heptis-B (Boryang, Korea), Amvax-B (Amson, Pakistan) and Engerix-B (GS & K, Belgium). To avoid complications related to multi dose vials, it was strictly followed that the vaccination dose for each subject should be company packed, individually in a sealed container and, formulated for intra muscular injection. The dosage vial should contain same amount i.e. 20 µgm/ ml of HBs Ag absorbed on to approximately 0.5 µgm / ml adjuvant (aluminum hydroxide) and 100 µgm / ml preservative (Thiomersal/ Thimerosal) in a final volume of 1.1 ml (1 dose/ vial). Storage temperature should maintain as 2°C to 8°C to ensure integrity. Participants

were injected with the vaccine of their own choice. Information brochures of the four vaccines were distributed among participants to help them make a choice. Out of total 729 doses administered to 243 individuals during this study, 195 were of Engerix-B, 420 were Heptis-B, 75 were Amvax-B, and 39 doses were of Euvax-B.

Categories for Determining Severity of an Adverse Effect:

- Local Symptoms: Soreness, indurations, swelling and redness.
- General Symptoms: Fever, headache and dizziness.
- Mild: Adverse events easily tolerated
- Moderate: Adverse event of sufficient discomfort to interfere with daily activity or requiring simple treatment (e.g. Paracetamol, Generic name: Paracip).
- Severe: Adverse event incapacitating and preventing usual activity or which may be life threatening, requiring hospitalization or completed treatment.

The course of an adverse event was described as:

- Spontaneous recovery without discontinuation of vaccination
- Recovery after discontinuation of vaccination
- Continuation of recovery after symptomatic treatment

Eight samples of Peripheral blood (2-3 ml) were taken from all vaccinees before administration of each dose, and at different intervals after completion of immunization as per schedule given below; the sera were collected and stored at - 20°C.

Table 1: Visits Were Scheduled As Follows:

	Vaccination number & sample collection
1.	First sample Before 1 st dose of Vaccine (Jan, 2003)
2.	Second sample Before 2 nd dose of Vaccine (Feb, 2003)
3.	Third sample Before 3 rd dose of Vaccine (June, 2003)
4.	Fourth sample After 6 months of 3 rd dose (Dec, 2003)
5.	Fifth sample After 15 months of 3 rd dose (Sep, 2004)
6.	Sixth sample After 19 months of 3 rd dose (Jan, 2005)
7.	Seventh sample After 22 months of 3 rd dose (May, 2005)
8.	Eighth sample After 30 months of 3 rd dose (Jan, 2006)

Antibody Estimation and Statistics:

Anti-HBs were detected by ELISA using IMX- Abbott and quantitated using appropriate dilution of a positive sample with a known concentration of anti-HBs expressed as IU/L, provided by the manufacturer. The assay determined IgG type of anti-HBs antibody and the protective level of antibody was considered >10 IU/L. *P* values of less than 0.05 were considered significant. On the whole Hepatitis B antibodies titer was determined in participants using five standards i.e. <10 IU/ml, Between 10 – 100IU/ml, Between 100 – 1000 IU/ml, >1000 IU/ml, and no response or no antibody titer for the period of 36 months starting Jan'2003 till Jan' 2006.

RESULTS:

Percentage of Geometric mean titer (GMT) of antibodies below 10 IU/ml was in between 0.0% to 0.22% in case of Engerix-B, 0.0% to 0.20% in case of Euvax B, 0.0% to 0.30% in case of Amvax-B and 0.0% to 0.25% in case of Heptis-B. Percentage of GMT of antibodies between 10 – 100 IU/ml was in between 0.0% to 10% in case of Engerix-B, 0.0% to 9% in case of Euvax B, 0.0% to 8.9% in case of Amvax-B and 0.0% to 9.3% in case of Heptis-B. Percentage of GMT of antibodies between 100 – 1000 IU/ml was in between 0.0% to 35% in case of Engerix-B, 0.0% to 30.1% in case of Euvax B, 0.0% to 39.3% in case of Amvax-B and 0.0% to 40% in case of Heptis-B. While, Percentage of GMT of antibodies above 1000 IU/ml was in between 0.0% to 23% in case of Engerix-B, 0.0% to 25% in case of Euvax B, 0.0% to 26% in case of Amvax-B and 0.0% to 23.7% in case of Heptis-B. When Percentage of GMT of negative response was calculated we found that on the whole Engerix-B showed no response after 6 months of 3rd dose in 0.9% recipients leading towards no response in 31.78% of recipients after 30 months of 3rd dose. Euvax-B showed no response after 6 months of 3rd dose in 7.9% recipients leading towards no response in 35.68% of recipients after 30 months of 3rd dose. Amvax-B showed no response after 6 months of 3rd dose in 11.6% recipients leading towards no response in 25.5% of recipients after 30 months of 3rd dose. While, Heptis-B showed no response after 6 months of 3rd dose in 9.88% recipients leading towards no response in 26.75% of recipients after 30 months of 3rd dose. Period after 6 months of 3rd dose and before 15th month of 3rd dose was the period when highest Percentage of GMT of anti- HBs was observed .

Local and generalized adverse effects observed during and after the immunization of volunteers were recorded separately for each vaccine (Table 2, 3, 4 & 5).

Table 2 Incidence of Local and/or Generalized Symptoms on Vaccination With Engerix-B

Dose	Total	Local Only		General only		Local & General		With symptoms	
	n	n	%	N	%	n	%	n	%
1	65	19	29	5	8	10	15	38	58
2	65	11	17	3	5	7	11	29	45
3	65	8	12	0	0	0	0	18	28
Total	195	38	19	8	4	17	9	85	44

Table 3 Incidence of Local and/or Generalized Symptoms on Vaccination With Euvax-B

Dose	Total	Local Only		General only		Local & General		With symptoms	
	n	n	%	n	%	n	%	n	%
1	13	4	31	2	15	3	23	8	62
2	13	2	15	1	0	2	15	6	46
3	13	2	15	0	0	0	0	0	0
Total	39	8	21	3	8	5	13	14	36

Table 4 Incidence of Local and/or Generalized Symptoms on Vaccination With Heptis-B

Dose	Total	Local Only		General only		Local & General		With symptoms	
	n	n	%	n	%	n	%	n	%
1	140	53	38	10	7	34	24	95	68
2	140	25	18	25	18	27	19	70	50
3	140	22	16	7	5	32	23	84	60
Total	420	100	24	42	10	93	22	249	59

Table 5 Incidence of Local and/or Generalized Symptoms on Vaccination With Amvax-B

Dose	Total	Local Only		General only		Local & General		With symptoms	
	n	n	%	n	%	n	%	n	%
1	25	13	52	8	32	3	56	23	92
2	25	2	8	6	24	4	16	9	36
3	25	14	56	3	12	25	100	12	48
Total	75	29	39	17	27	32	43	44	59

As Shown in Table 6 that one month after the first dose, 180/243 subjects (74.45%) had seroprotection with respect to anti-HBs. One month after the second dose, 189/243 subjects (77.92%) showed seroprotection, four months after second dose, at month 6, 224/243 subjects (92.43%) were seroprotective. After 15 months of 3rd dose 231/243 subjects (95.27%) showed maximum seroprotection level in the immunized women. Later on seroprotection level was determined after 19, 22 and 30 months of 3rd dose. 170/243 subjects (70.08%) subjects were seroprotected even after 30 months of 3rd dose without having any booster dose.

Table 6: Over All Seroprotection Levels and Geometric Mean Titers (GMT) of Anti- HBs Antibodies

	Timing	N	S*	% of S*	GMT	Range of Anti HBs Titer
PRE	Before Vaccination (Jan, 03)	243	0	0	0	0
M1	Before 2nd dose (Feb, 03)	243	180	74.45	16	0-50
M2	Before 3rd dose (June, 03)	243	189	77.92	143	1-700
M3	After 6 months of 3rd dose(Dec, 03)	243	224	92.43	18500	45-50000
M4	After 15 months of 3rd dose(Sep, 04)	243	231	95.27	23000	40-50000
M5	After 19 months of 3rd dose(Jan, 05)	243	222	91.57	12100	39-33000
M6	After 22 months of 3rd dose(May, 05)	243	195	80.53	1875	5-17000
M7	After 30 months of 3rd dose(Jan, 06)	243	170	70.08	690	1-1500

Comparative results of serum protection analysis of 04 candidate vaccines determined after 15 months of third dose without giving any booster dose, were found effective in healthy young female volunteers, demonstrating induction of very good immunogenicity. No significant differences were observed in seroprotection level of test vaccines (Table7). Overall serum

protection rate achieved in case of Engerix-B was 95.9%, in case of Euvax-B, it was 95.2%, in case of Heptis-B was 95.0% , and in case of Amvax-B it was 95.1%. Calculated P values for all four test vaccines were lesser than 0.05 indicating significance of the used vaccines.

Table 7: Comparative Serum Protection analysis of 04 different Hepatitis-B Vaccines(n= 243; mean age= 21.5+3.7 Years)

Name of Vaccine	Mean Serum Protection Level 10µgm x 3	95% Confidence Level	P Value
Engerix-B (n=65)	95.9%	94.5% - 100%	<0.05
Euvax-B (n=13)	95.2%	94.3% - 100%	<0.05
Heptis-B (n= 140)	95.0%	92% - 99.4%	<0.05
Amvax-B (n= 25)	95.1%	92% - 98%	<0.05

DISCUSSION:

As yet no such immunogenicity trials have been conducted in Pakistan or risk factors indigenous to the region assessed for any of the vaccine being utilized at the population level for such long period. Our study, is one of the longest study, conducted between Jan'2003 and Jan' 2006, which includes total 243 healthy women subjects of child bearing age group. In this study we have demonstrated that there is no significant difference in reactogenicity and serum protection level among all four candidate vaccines we tested here, excellent immunogenicity of vaccines in volunteers recommends their usage for immunization purpose among different communities without having any doubts related to reactogenicity and side effects.

Recombinant hepatitis B vaccines have long been used for protection in the serum of and three doses have been shown to produce Anti HBs in the serum of approximately 95% of people who have not encountered the virus. The antibody response declines with increasing age. Patients older than 30 years have an increased risk of no response to HBV vaccine, as compared with younger persons¹⁷. Thus, immunization during childhood or adolescence offers the greatest potential for protection¹⁷ and provides lifelong immunity. Ninety percent of healthy adults and 95 percent of infants, children, and adolescents have protective serum anti-HBs antibody concentrations after the vaccine series has been completed¹⁸.

Two kinds of recombinant vaccine are used for active immunization against hepatitis B; one of them contains the PreS1 and PreS2 antigenic domains while the other kind contains S and PreS polypeptide. No important differences between the effectiveness of these two types of vaccine have been detected¹⁹. In a series of studies it has been demonstrated that 90-99% of healthy neonates, children, adolescents and adults develop protective levels of anti-HBs antibody following a standard vaccination course with hepatitis B vaccine^{20, 21, 22, 23, 24, 25}.

Efficacy of vaccines in the field have been measured long after the vaccine have been introduced at large scale population levels and only selected countries have record keeping such as the Centers for Disease Control in USA and the National Health Services in UK. Most developing countries do not have infra structure to support these activities and therefore the efficacy and risk indigenous to the population remains unknown.

Procurement and delivery of high-quality vaccine has national and international public health and 'public good' implications far beyond the scope of most products. People immunized with vaccines of inadequate quality can become ill and die from the disease that the vaccine should have prevented. Even more lives are placed at risk if vaccination coverage declines as a result of reduced public confidence in immunization programs. If we look at the outcome of immunization programs in different countries then we will have a good idea that how mass vaccination helped in reduction of disease burden?²⁶

Importance of dose size, number of doses and dose response is another important issue related to immunization programs. Published studies regarding the dose-response relationship in terms of immunogenicity and sero-protection are highly varied. Chiamonte *et al*²⁷ reported that the sero-protection reached a level of 99.6% within one month after primary immunisation with the recombinant hepatitis B vaccine. The findings of Assateerawatt *et al*²⁸ and Just *et al*²⁹ also were the same. Baldy JLS *et al* carried out a comparative study with three recombinant hepatitis B vaccines, one Brazilian (Butang, Instituto Butantan) and two Korean vaccines (Euvax-B, LG Life Sciences Ltd. and Hepavax-Gene, Green cross Vaccine Corp.), administered intramuscularly to students aged 17 to 19 years in three doses (corresponding to half the amount of antigen routinely used for adult vaccination) at intervals of one month between the first and second dose, and of four months between the second and third dose. The GMT of anti-HBs induced by the Euvax-B and Engerix-B vaccines were higher than those obtained with the Butang vaccine ($p < 0.05$); this difference was not significant when comparing the other vaccines two-by-two. No spontaneous adverse effects attributable to the application of any dose of the three vaccines were reported³⁰.

Vaccine efficacy is defined as the reduction in the incidence of a disease among people who have received a vaccine compared to the incidence in unvaccinated people. The efficacy of a vaccine is measured in clinical trials by giving one group of people a vaccine and comparing the incidence of disease in that group to another group of people who do not receive the vaccine. In our study overall efficacy of the vaccines used was satisfactory, without producing severe adverse effects, also there is no report of incidence of disease till now, in those who were vaccinated during this study (Table 2, 3, 4, 5).

Maximum protection level in terms of immunogenicity was observed in Euvax-B, which showed GMAT of 35.68% in test population. Engerix-B showed GMAT of 31.78%, Hepatitis-B

showed GMAT of 26.75% and, Amvax-B showed GMAT of 25.5% after 30 months of 3rd dose of immunization. While highest serum protection level was achieved in case of Engerix-B i.e. 95.9%. On the whole, r-hepatitis B vaccines showed high immunogenicity and good safety profile in the test population.

The inclusion of Hepatitis B in the list of compulsory and Extended Program for Immunization (EPI) in Pakistan since 2005 will result in mass vaccination of pediatric population. However, a big chunk of the adult population, especially healthcare workers, also needs to be immunized against Hepatitis B infection. We believe that all of the above mentioned HB vaccines, which are easily available in Pakistan's market can be used for these mass vaccination programs without having any doubts related to Reactogenicity and Immunogenicity.

CONCLUSION:

In conclusion, this prospective study reinforces that the four different recombinant hepatitis B vaccines licensed in Pakistan have a good tolerability and are highly immunogenic among young women. It is also recommended that government should ensure the serosurvey of HBsAg and vaccine coverage at country level in order to reduce the disease burden on country's economy.

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COMPETING INTERESTS

None Declared

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