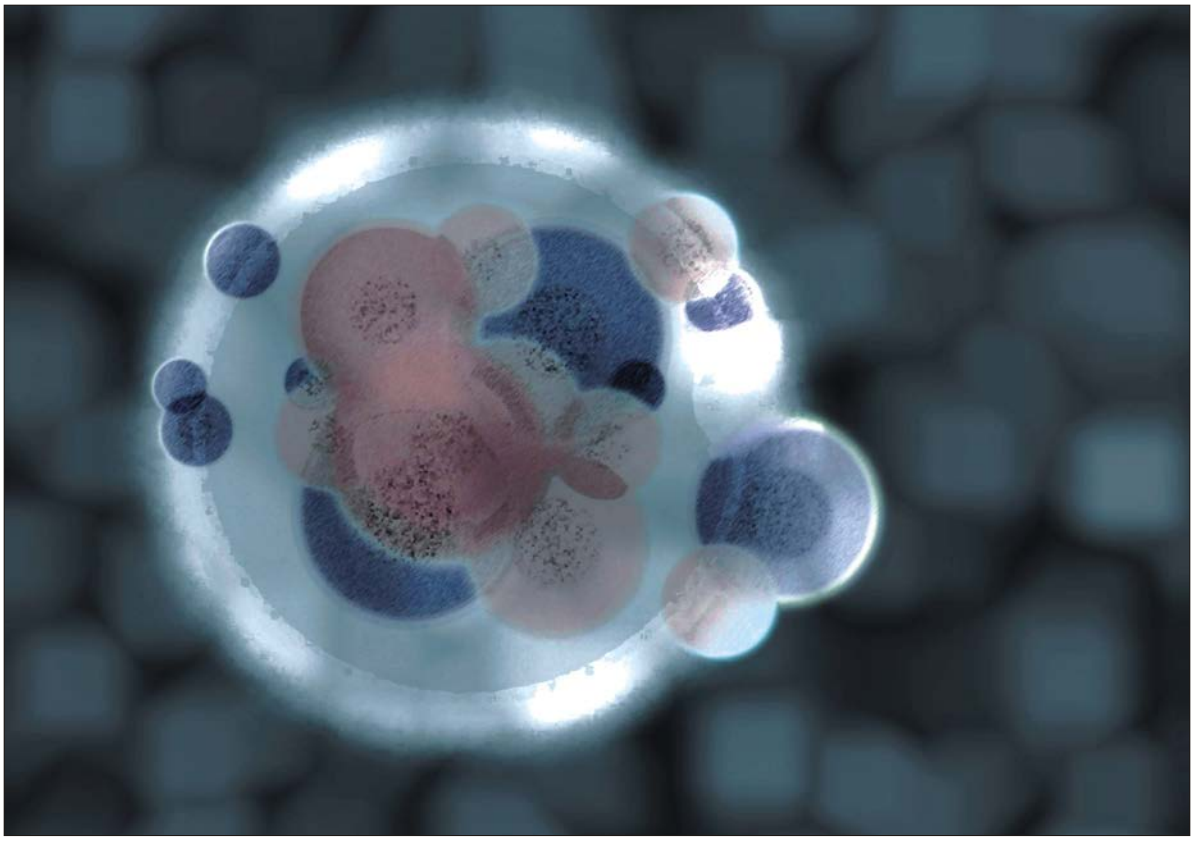

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Comparative Immunogenicity of Commercially Available Recombinant Vaccines Against Hepatitis B in Human Urban Population of Bahawalpur District, Pakistan

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Abstract: The immunogenicity profiles of the recombinant hepatitis B vaccines commercially available in Pakistan were compared to Engerix-B® in a double-blind, prospective equivalence study. A total of 744 subjects (449 male, 295 female) of different age group and professions (randomly selected) of urban areas of Bahawalpur district were included in this study. Population was divided in six different groups (children, young and adult of either sex) and each group was administered (intramuscularly) one brand of vaccine (0, 1 and 6 month schedule). Children groups were vaccinated by 10 μg while young and adults groups by 20 μg of respective vaccine. The participants had blood samples taken (3-times; at the time of second dose, one and six months after third dose). The seroprotection; geometric mean titer (mIU mL^{-1}) of six different vaccine used, were found maximum 1880.46±12.48 with Amvax B and minimum 1790.62±18.26 with Hepa-B-vac in children group, from 1897.43±17.26 (Engirex-B) to 1815.46±12.74 (Hepa-B-vac) in young group and from 1881.75±10.97 (Amvax-B®) to 1729.82±8.85 (Hepa-B-vac) in adult group. The antibody titers <10 mIU mL^{-1} were found highest (3.8%) with Hepa-B-vac® and lowest (2.4%) with Amvax-B® and Hepavax-Gene®. Antibody titers 10<100, 100<1000 and >1000 mIU mL^{-1} were observed 14.56 (Hepa-B-vac®) to 9.1% (Amvax-B®), 40.56 (Heberbiovac) to 39.15% (Hepavax-Gene) and 48.8 (Engirex-B) to 41.61% (Hepa-B-vac), respectively. The study concluded that all the recombinant hepatitis B vaccines tested were equipotent and similar in immunogenicity to Engerix-B® in the human urban population of Bahawalpur-Pakistan.

Key words: Hepatitis B, hepatitis B vaccine, recombinant vaccines, immunization

INTRODUCTION

The invasion of Hepatitis B Virus (HBV) causes Hepatitis-B that has been considered a serious global health hazard with three quarters of world's population living in areas where prevalence of HBV infection is 2% or more. About 400 million individuals are chronic carriers globally, of this 25-30% will die as a consequence of the infection. HBV can cause a life-long infection, which can further lead to cirrhosis of the liver, hepatic cancer, hepatic failure and eventually death (Chuanfang *et al*., 2006; Rifat-uz-Zaman, 2006).

HBV has blamed for 5000 deaths annually. Prevalence is low in persons younger than 12 years, but it increases in those older than 12 years. The increased prevalence in persons older than 12 years associates with the initiation of sexual contact (the major mode of transmission), the number of sexual partners and an early age of first intercourse. The cocaine use, high number of sexual partners, divorced or separated marital status, foreign birth and low educational level remain some other means (CDC, 2005).

Unfortunately the Hepatitis-B carrier rate in Pakistan has also been alarmingly high i.e., approximately 10%. Both the acute and chronic hepatitis B virus infections cause major health problems (Rifat-uz-Zaman, 2006). The simple way to guard against this dreadful disease is by vaccination (Szmuness *et al*., 1980).

Two types of vaccines for hepatitis B have been indicated. One is derived from plasma (plasma derived vaccine) and the other is derived from yeast or mammalian cells (recombinant vaccine) (Assad and Francis, 1999). Repeated injections over months are required to mount an effective antibody response with vaccination. Hepatitis B immunoglobulin has high levels of antibody to hepatitis B surface antigen. The immunoglobulin is immediately effective and seems protective for several months, after which it wanes (Beasley and Hwang, 1983; Nair *et al*., 1984). Experience with the yeast recombinant hepatitis B vaccine now exceeds more than 10 years. Many reviewers mentioned about vaccination and showed the vaccines to be safe, causing mostly only minor local symptoms and to be highly immunogenic both in monitored clinical trials and under field conditions. Most of the vaccines consistently elicited high geometric mean antibody titers (GMT) and a high protective efficacy has been established in population at high-risk of hepatitis B infection (Assad and Francis, 1999).

In the present study, we assessed the comparative efficacies of commercially available hepatitis B vaccines for the sake of public interests.

MATERIALS AND METHODS

Study design: This was a double-blind, prospective equivalence study in the urban population of district of Bahawalpur-Pakistan to assess and compare the immunogenicity of hepatitis B recombinant vaccines (20 μg mL^{-1}) commercially available in Pakistan to vaccine, Engerix-B®, (20 μg mL^{-1}) an active control.

The present study was carried out from Feb. 01, 2004 to September 14, 2005. A total of 744 peoples (449 male, 295 female) of different profession and age groups of urban areas of Bahawalpur-Pakistan were included.

Healthy individuals of either sex, age (1-45 years) without any previous hepatitis B vaccination was included in this study. The population was divided into six different groups and each group was further subdivided into three age groups i.e., mature male/female (age 17-45 years), young male/female (age 10-17 years) and children male/female (age 1-10 years). A willingness certificate for cooperation in carrying out the purpose of present study was obtained, singed by each individual/parents/guardians before his/her/children inclusion in the study (Martins *et al*., 2005).

Inclusion and exclusion criteria: Only healthy subjects who informed not having received previous hepatitis B vaccination were eligible for the study. Candidates who presented a positive or inconclusive HBV serological marker in the first blood sample or were anti-HBc positive after the third dose were excluded from the analysis of adherence to protocol (Reinaldo *et al*., 2004).

Full compliance to the study protocol was defined by the following criteria: providing a blood sample for serology before the first dose; receiving three doses of vaccine; interval between the first two doses ≥ 28 days and ≤ 90 days, interval between the second and third doses of at least 120 days, interval between the third dose and final blood sample ≥ 28 days and ≤ 180 days and blood sample and serology after the third dose (Reinaldo *et al*., 2004; Martins *et al*., 2005).

Vaccine: The following recombinant hepatitis B vaccines were included in the study: Amvax-B (Chiron Biocine/Amson), Heberbiovac® (Heber Biotec/Mecter), Hepavax-Gene® (Greencross Vaccine Corp., Korea), Hepa-B-vac® (Shenzhen/R.Y. International) and Heptis-B® (Boryung Biopharma Co., Ltd., Korea) for comparison of the results, Engerix-B® (GlaxoSmithKline, England), whose immunogenicity in individuals of different age groups has already been demonstrated (Bryan *et al*., 1995; Leroux-Roels *et al*., 2000; Schiff *et al*., 1995).

Vaccine administration: Vaccine administration was intramuscular in the deltoid (mature, young and children). Needle size was chosen according to fat thickness at the injection site. In general, the needles used were 24 G 3/4 in children and 22 G 1 or 22 G 1 1/4 in young and matures. For all vaccines, a dose of 10 μg (0.5 mL) was administered to children and 20 μg (1 mL) to young and matures. The recommended schedule was 0, 1 and 6 months (Reinaldo *et al*., 2004; Martins *et al*., 2005).

Specimen collection: Six milliliter of fresh blood sample was withdrawn from each subject by vene-puncture arm vein. Serological analysis was conducted on blood samples drawn on three occasions: just prior to the first vaccine dose, from 28-32 days and 180 ≤ days after the third and final dose (Reinaldo *et al*., 2004; Martins *et al*., 2005).

Sample analysis: Serum samples collected before the first dose were screened for markers of hepatitis B virus infection: HBsAg, anti-HBs and anti-HBc using a microparticle enzyme immunoassay (MEIA; Axsym Abbott). One and six months after application of the third dose of vaccine, a second and third venous blood samples were collected from all vaccinees for the separate determinations of the same serological markers of hepatitis B virus infection (HBsAg, anti-HBs and anti-HBc) by the same technique (MEIA, Axsym Abbott). Anti-HBs titers were interpreted based on data reported in the literature (Francis *et al*., 1982; Hadler *et al*., 1986): <10 mIU mL^{-1} = non-reactive (non-responders); >10 mIU mL^{-1} = reactive; 10 to 100 mIU mL^{-1} = reactive (poor responders) and >100 mIU mL^{-1} = reactive (good responders) (Martins *et al*., 2005).

Data analysis: All data were expressed as means±standard deviation (SD). The Student t test was used to analyze the significance of difference. A p-value of <0.001 was considered significant (Sanders, 1990).

RESULTS

The present study included a total of 900 individuals; 500 male (55.56%) and 400 female (44.45%) of different age groups and professions of urban areas of Bahawalpur district-Pakistan. Of which the 744 individuals (449 male; 60.35%, 295 female; 39.65%) completed the serological tests (Table 1).

Results obtained with the *Engirex-B*® vaccine: The interval between application of the first and second *Engirex-B*® dose and collection of first blood sample ranged from 28 to 35 days (30.30±1.86), the interval

Table 1: Population distribution according to the vaccine administered

	Vaccinated population					
	Female		Male		Total	
Vaccine used	No.	(%)	No.	(%)	No.	(%)
Engirex-B®	49	16.61	75	16.70	124	16.67
Amvax-B®	51	17.29	79	17.59	130	17.47
Heberbiovac®	49	16.61	76	16.93	125	16.80
Hepa-B-vac®	47	15.93	78	17.37	125	16.80
Hepavax-Gene®	49	16.61	66	14.71	115	15.46
Heptis-B®	50	16.95	75	16.70	125	16.80
Total	295	100.00	449	100.00	744	100.00

between application of the second and third dose ranged from 150 to 155 days (152.43±1.94) and the interval between application of the third dose and collection of second blood sample ranged from 32 to 34 days (32.8±1.19). The third sample was collected at the interval of 180 to 190 days (184.42±2.46) post-third dose of vaccine. GMT of anti-HBs in population who received this vaccine was 1912.36±18.57 in children, 1870.14±11.46 in young and 1997.43±17.26 in adult groups.

Results obtained with the *Amvax-B*® vaccine: The interval between application of the first and second *Amvax-B*® dose and collection of first blood sample ranged from 29 to 37 days (32.30±2.10), the interval between application of the second and third dose ranged from 148 to 154 days (151.20±2.14) and the interval between application of the third dose and collection of second blood sample ranged from 30 to 34 days (31.8±1.57). The third sample was collected at the interval of 185 to 192 days (187.20±3.63) post-third dose of vaccine. GMT of anti-HBs in population who received this vaccine was 1880.46±12.48 in children, 1981.75±10.97 in young and 1946.24±14.29 in adult groups.

Results obtained with the *Heberbiovac*® vaccine: The interval between application of the first and second *Heberbiovac*® dose and collection of first blood sample ranged from 27 to 38 days (31.84±2.43), the interval between application of the second and third dose ranged from 154 to 159 days (155.12±2.40) and the interval between application of the third dose and collection of second blood sample ranged from 28 to 33 days (30.42±2.15). The third sample was collected at the interval of 184 to 194 days (188.46±2.24) post-third dose of vaccine. GMT of anti-HBs in population who received this vaccine was 1778.25±16.58 in children, 1727.78±6.46 in young and 1816.18±16.27 in adult groups.

Results obtained with the *Hepa-B-vac*® vaccine: The interval between application of the first and second *Hepa-B-vac*® dose and collection of first blood sample ranged from 29 to 35 days (31.23±1.87), the interval between application of the second and third dose ranged from 149 to 153 days (152.35±2.32) and the interval between application of the third dose and collection of second blood sample ranged from 29 to 36 days (33.2±2.43). The third sample was collected at the interval of 188 to 193 days (188.26±2.88) post-third dose of vaccine. GMT of anti-HBs in population who received this vaccine was 1710.62±18.26 in children, 1729.82±8.85 in young and 1845.46±12.74 in adult groups.

Results obtained with the *Hepavax-Gene*® vaccine: The interval between application of the first and second *Hepavax-Gene*® dose and collection of first blood sample ranged from 30 to 36 days (33.20±2.54), the interval between application of the second and third dose ranged from 154 to 160 days (155.47±2.63) and the interval between application of the third dose and collection of second blood sample ranged from 29 to 36 days (32.6±2.13). The third sample was collected at the interval of 185 to 193 days (188.43±2.74) post-third dose of vaccine. GMT of anti-HBs in population who received this vaccine was 1810.65±19.46 in children, 1814.46±9.65 in young and 1868.27±10.45 in adult groups.

Results obtained with the *Heptis-B*® vaccine: The interval between application of the first and second *Heptis-B*® dose and collection of first blood sample ranged from 27 to 35 days (30.53±2.46), the interval between application of the second and third dose ranged from 148 to 159 days (154.12±2.75) and the interval between application of the third dose and collection of second blood sample ranged from 30 to 35 days (32.8±1.74). The third sample was collected at the interval of 186 to 192 days (188.36±2.45) post-third dose of vaccine. GMT of anti-HBs in population who received this vaccine was 1901.74±17.54 in children, 1909.09±7.16 in young and 1862.32±13.74 in adult groups.

DISCUSSION

No investigations are available in the literature evaluating the immunogenicity of the five recombinant vaccines studied in Pakistan using 0, 1 and 6 month scheme in children (10 μg), young and adults (20 μg doses each). The immunogenicity of two or three recombinant hepatitis B vaccines, including the *Engerix-B*® (GlaxoSmithKline, England), administered in three doses intramuscularly has been demonstrated in children, young and adults (Catania *et al*., 1996; Leroux-Roels *et al*., 2000) and their use has been recommended by American Academy of Pediatrics (2000).

Table 2: Comparative immunogenicity of different vaccines in children (age; 1≥10 years)

Vaccine	No.	Mean Anti-HBs titer (mIU mL^{-1}) after			Geometric mean titer (GMT)
		1 month of 1st dose	1 month of 3rd dose	6 month of 3rd dose	
Engirex-B®	33	8.62±0.637	707.37±10.17	506.36±13.12	1872.36±18.57
Amvax-B®	40	8.35±0.461	712.72±12.32	509.42±12.21	1880.46±12.48
Heberbiovac®	36	7.35±0.634	678.02±11.52	501.47±11.24	1828.25±16.58
Hepa-B-vac®	34	6.28±0.546*	664.46±10.79*	469.51±12.87	1790.62±18.26*
Hepavax-Gene®	31	8.25±0.763	712.72±11.88	507.72±13.32	1818.65±19.46
Heptis-B®	34	8.43±0.678	698.17±10.55	497.43±11.38	1821.74±17.54

Test vaccines: Significant from Engirex-B * p<0.001, All the other values are NS (p>0.001) from Engirex-B, Mean±SEM = Mean values±Standard Error of Means

Table 3: Comparative immunogenicity of different vaccines in young (age; 10≥17 years)

Vaccine	No.	Mean Anti-HBs titer (mIU mL^{-1}) after			Geometric mean titer (GMT)
		1 month of 1st dose	1 month of 3rd dose	6 month of 3rd dose	
Engirex-B®	47	8.95±0.680	707.37±10.17	506.36±8.12	1897.43±17.26
Amvax-B®	46	8.35±0.463	712.72±10.32	509.42±9.21	1856.24±14.29
Heberbiovac®	46	8.73±0.577	735.02±7.520	511.47±7.24	1846.18±16.27
Hepa-B-vac®	44	6.35±0.631*	681.46±6.790	507.72±9.32	1815.46±12.74*
Hepavax-Gene®	43	8.20±0.724	712.72±09.88	489.51±9.87	1868.27±10.45
Heptis-B®	45	8.82±0.718	698.17±08.55	487.43±8.38	1862.32±13.74

Test vaccines: Significant from Engirex-B * p<0.001, All the other values are NS (p>0.001) from Engirex-B, Mean±SEM = Mean values±Standard Error of Means

In the present study, the immunogenicity of five vaccines (Amvax-B®, Heberbiovac®, Hepa-B-vac®, Hepavax-Gene® and Heptis-B®), administered intramuscularly to non-serological randomly selected peoples, both male and female of different ages and professions, was demonstrated (Table 1). The seroprotective levels of all the vaccines under test were compared with the standard vaccine, Engirex-B®.

Engirex-B yielded the antibody titres, 8.62±0.637, 707.37±10.17, 506.36±13.12 mIU mL^{-1} after one month of 1st dose, after one month and six months of 3rd dose, respectively in the children group. Amvex-B, Heberbiovac®, Hepavax-Gene®, Heptis-B® were established similar (different insignificantly) antibody titers in the respective groups of children, however, Hepa-B-vac® showed significantly (p<0.001) lesser antibody titres in children one month after the 1st and 3rd doses. Additionally GMT following the administration of Amvex-B®, Heberbiovac®, Hepavax-Gene®, Heptis-B® were not found dissimilar in comparison to the Engirex-B®, except Hepa-B-vac® which caused significantly (p<0.001) lesser GMT (Table 2). The data indicated some what slower onset of imunogenicity caused by Hepa-B-vac®. However the immunogeicity developed by all other vaccines under trial found comparable to the Engirex-B in children groups (Table 2).

The young and adult groups of the population under test were also indicated no significant differences in the immunogenicity (antibody titers) caused by vaccines like Amvex-B, Heberbiovac®, Hepavax-Gene® and Heptis-B® in comparison to Engirex-B after one month of 1st and 3rd doses as well as six months of 3rd dose. Hepa-B-vac® however, established significantly different (p<0.001) seroprotection one month after 1st dose in young and one month after 3rd dose in adult groups. Geometric Mean Titers (GMT) of Amvex-B®, Heberbiovac®, Hepavax-Gene® and Heptis-B® were observed not different significantly in the whole population under test in comparison to the GMT of Engirex-B®, except Hepa-B-vac® (significantly lesser GMT in children, young and adult groups). The findings confirmed the slow rate of immunogenicity with Hepa-B-vac® in comparison to all the vaccine used including Engirex-B® (Table 3 and 4).

Figure 1 detailed on percent of the subjects with below 10, between 10 and 100, between 100 and 1000 and above 1000 mIU mL^{-1} of antibody titers after six months of 3rd doses of all the vaccines used in the study. Engirex-B®, Amvex-B®, Heberbiovac®, Hepavax-Gene®, Heptis-B® and Heptis-B® yielded 2.6, 2.4, 2.5, 3.8, 2.4 and 3.1%, (respectively) antibody titers below 10 mIU mL^{-1} in the population. The vaccines caused 9.4, 9.1, 11.4, 14.56, 9.9% and 10.2%, (respectively) antibody titers between 10-100 mIU mL^{-1}; 39.2, 40.12, 40.56, 40.13, 39.15 and 39.9%, (respectively) antibody titers between 100-1000 mIU mL^{-1}; 48.8, 48.38, 45.54, 41.61, 48.55 and 46.8, (respectively) antibody titers above 1000 mIU mL^{-1} in the population under trial. The data clearly indicated that all the vaccines successfully caused the antibody titers levels above 100 mIU mL^{-1} (non/poor-responders) (Baldy *et al*., 2004; Chuanfang *et al*., 2006), in the higher fractions of population and lesser fractions remained below

Table 4: Comparative immunogenicity of different vaccines in matures (age; 17≥45 years)

Vaccine	No.	Mean Anti-HBs Titer (mIU mL^{-1}) after 1 month of 1st dose	1 month of 3rd dose	6 month of 3rd dose	Geometric mean titer (GMT)
Engirex-B®	44	8.35±0.746	688.77±10.41	510.36±8.410	1870.14±11.46
Amvax-B®	44	8.13±0.541	702.13±10.73	503.58±09.72	1881.75±10.97
Heberbiovac®	43	7.32±0.764	660.99±10.74	500.66±10.74	1847.78±6.460
Hepa-B-vac®	47	7.58±0.655	611.14±11.26*	498.48±13.47	1729.82±8.850*
Hepavax-Gene®	41	7.25±0.473	710.35±11.36	502.83±11.48	1834.46±9.650
Heptis-B®	46	8.14±0.528	703.45±12.62	504.51±10.73	1839.09±7.160

Test vaccines: Significant from Engirex-B * p<0.001, All the other values are NS (p>0.001) from Engirex-B, Mean±SEM = Mean values±Standard Error of Means

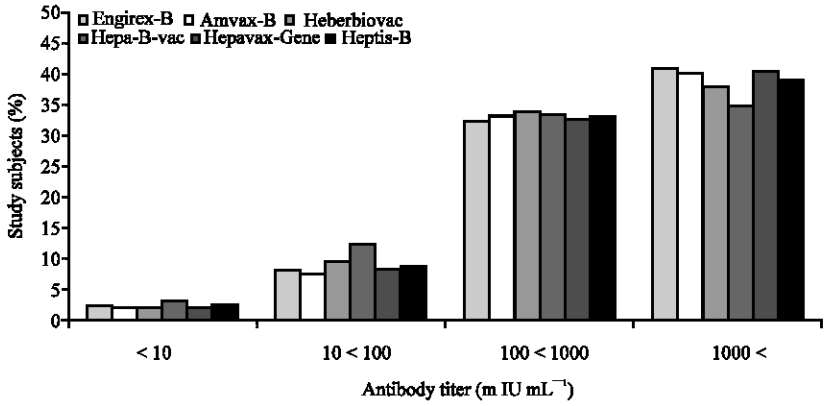

Fig. 1: Comparative antibody titer of different recombinant hepatitis B vaccines six months after their third doses

100 mIU mL^{-1} (good-responders) (Baldy *et al*., 2004; Chuanfang *et al*., 2006) even after six months of the completion of the vaccination against hepatitis B (Fig. 1). Hepa-B-vac® caused significantly lesser immunogenicity in comparison to Engirex-B® in population under trail but it showed antibody titers 96.2% above 100 mIU mL^{-1} and 3.8% below in comparison 97.4% above and 2.6% below 100 mlU mL^{-1} by Engirex-B®. Therefore results remained comparable. The data was also found comparable with findings observed by Leroux-Roels *et al*. (2000) and Schiff *et al*. (1995) who made comparison of two commercial recombinant vaccines for hepatitis B in adolescents (Martins *et al*., 2005).

The data further indicated the maximum antibody titers were established one month after with the administration of 3rd doses of all the vaccine under test which were followed by antibody titers determined, six months after the applications of 3rd/final doses of different vaccines in the separate groups of children, young and adults (Table 2-4). However, immunogenicity caused by Hepa-B-vac® was found in a slow but sustained manner in comparison to the all other vaccines. Moreover, Amvax-B®, Heberbiovac®, Hepavax-Gene® and Heptis-B® showed similar seroprotection against hepatitis B to that of Engirex-B®.

REFERENCES

American Academy of Pediatrics, 2000. Hepatitis B. In: Pickering, L.K. (Ed.), 2000 Red Book: Report of the Committee on Infectious Diseases. 25th Edn., Elk Grove Village, American Academy of Pediatrics, 294.

Assad, S. and A. Francis, 1999. Over a decade of experience with a yeast recombinant hepatitis B vaccine. Vaccine, 18: 57-67.

Baldy, J.L., G.Z. de Lima, H.K. Morimoto, E.M. Reiche, T. Matsuo, E.D. de Mattos and L.C. Sudan, 2004. Immunogenicity of three recombinant hepatitis B vaccines administered to students in three doses containing half the antigen amount routinely used for adult vaccination. Rev. Inst. Med. Trop. Sao Paulo, 46: 103-107.

Beasley, R.P. and L.Y. Hwang, 1983. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J. Infect. Dis., 147: 185-190.

Bryan, J.P., P.G. Craig and L. Reyes *et al*., 1995. Randomized comparison of 5 and 10 microgram doses of two recombinant hepatitis B vaccines. Vaccine, 13: 978-982.

Catania, G., V. Di Ciommo and C. Concato, 1996. Vaccination against hepatitis B virus in children and adolescents in a pediatric hospital. Recent Prog. Med., 87: 271-274.

CDC, 2005. Transmission of hepatitis B virus among persons undergoing blood glucose monitoring in long-term-care facilities-Mississippi, North Carolina and Los Angeles County, California. MMWR, 54: 220-223.

Chuanfang, L., G. Yan, B. Jesper, B.H. Elizabeth and G. Christian, 2006. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: Systematic review and meta-analysis. BMJ., 332: 328-336.

Francis, D.P., S.C. Hadler and S.E. Thompson *et al*., 1982. The prevention of hepatitis B with vaccine. Report from the Centers for Disease Control multi-center efficacy trial among homosexual men. Ann. Intl. Med., 97: 362-366.

Hadler, S.C., D.P. Francis and J.E. Maynard *et al*., 1986. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. New Eng. J. Med., 315: 209-214.

Leroux-Roels, G., B. Abraham, M. Fourneau, N. De Clercq and A. Safary, 2000. A comparison of two commercial recombinant vaccines for hepatitis B in adolescents. Vaccine, 19: 937-942.

Martins, R.M., G. Bensabath, L.C. Arraes, L. Oliveira-Mde, J.C. Miguel, G.G. Barbosa and L.A. Camacho, 2005. Multicenter study on the immunogenicity and safety of two recombinant vaccines against hepatitis B. Mem Inst Oswaldo Cruz, 99: 865-871.

Nair, P.V., J.Y. Weissman, M.J. Tong, M.W. Thursby, R.H. Paul and C.E. Henneman, 1984. Efficacy of hepatitis B immune globulin in prevention of perinatal transmission of the hepatitis B virus. Gastroenterology, 87: 293-298.

Reinaldo, M.M., B. Gilberta, A.C. Luiz, O.A.L. Maria de, M.C. Juliana, B.G. Glayse and C.B.A. Luiz, 2004. Multicenter study on the immunogenicity and safety of two recombinant vaccines against hepatitis B. Mem. Inst. Oswaldo Cruz, 999: 865-871.

Rifat-uz-Zaman, 2006. Prevalence of Hepatitis B and Hepatitis C viruses in human urban population of Bahawalpur District, Pakistan. J. Med. Sci., 6: 429-435.

Sanders, D.H., 1990. Statistics. A Fresh Approach, 4th Edn. Auckland: McGraw-Hill Book Company.

Schiff, G.M., J.R. Sherwood, J.B. Zeldis and D.S. Krause, 1995. Comparative study of the immunogenicity and safety of two doses of recombinant hepatitis B vaccine in healthy adolescents. J. Adolesc. Health, 26: 12-17.

Szmuness, W., C.E. Stevens, E.A. Zang, E.J. Harley and A. Kellner, 1981. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): A final report. Hepatology, 1: 377-385.