Long-term Immunogenicity of Hepatitis B Vaccination in children

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ABSTRACT

Background: Chronic hepatitis B is a major global healthcare problem. Immunization is the most effective way to prevent transmission of hepatitis B virus (HBV) and, hence, the development of acute and chronic hepatitis B. Sero-protection after vaccination, defined as anti hepatitis B surface antibody (anti-HBS) ≥ 10mIU/mL, is achieved in over 95% of all vaccinated children.
Objective: The aim of the work is to detect the long-term immunogenicity of the vaccine in children after five and ten years of vaccination, also to test for anamnestic reaction to determine whether or not a booster dose is needed.
Methods: This study included 200 healthy children. Before being included in the study children were screened for the presence of HBV infection. The children were divided into two groups according to age (each group contains 100 children). Their data are included. Group `A` included 53 males and 47 females, around 6 years old, all children were vaccinated 5 years ago. Group `B` included 27 males and 73 females, around 11 years old. All children vaccinated 10years ago. HBsAb titre was tested in their blood, booster dose of the vaccine was given to children whose HBsAb was < 10 mIU/ml, then one and half months later, another blood sample from each of them was retested for HBsAb to evaluate the response to this booster dose of vaccine.
Results: when testing the serum of the children in both groups (A&B) for HBsAb and its titre, both groups have a wide range concerning the level of HBsAb (2→1000) mIU/ml). Our data proves the decline of antibodies titre with time, and there was significant difference between the two groups in the level of HBsAb . There was no significant difference in anti-HBs between girls and boys in group A in contrary to group B. In group A, from the nineteen children who needed a booster vaccination dose, 14 were vaccinated. Serum sample was taken from 10 children after one and half month from vaccination, out of these 10, 9 (90%) responded by increased level of HBs antibodies and only one child did not respond, Six (66.6%) of the nine showed an adequate response. In group B, fifty two children in this group had antibody titre < 10, forty eight were vaccinated. After one and half month, 34 children were tested again for HBsAb. Two out of the thirty four children did not respond (5.8%) and 32 (94.2%) responded by an increase in the antibody titre. Of those responded, 19 had adequate response (HBsAb ≥ 100) and 13 had hypo-response (HBsAb lies between 10-100). Therefore, 80% of the boys who were retested for HBsAb after vaccination responded adequately while 51.7% of the corresponding girls responded adequately. There was no significant difference in antibodies titre responding to the testing dose with p value =0.814.

Conclusion: Hepatitis B vaccine is an effective and successful way for preventing HBV infection. There is persistence of protective antibodies after primary vaccination in most of children with decline of the levels over time. Even so, no need for booster dose at least for 10 years after vaccination **.**

Key words: HBV, HB Vaccination, child immunity.

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INTRODUCTION:

Chronic hepatitis B is a major global healthcare problem ***(1)***. Immunization is the most effective way to prevent transmission of hepatitis B virus (HBV) and, hence, the development of acute and chronic hepatitis B. Sero-protection after vaccination, defined as anti hepatitis B surface antibody (anti-HBS) ≥ 10 mIU/mL, is achieved in over 95% of all vaccinees ***(2).*** Widespread immunization programs against HBV, which have been implemented in more than 100 countries, have dramatically reduced the occurrence of chronic HBV infection and hepatocellular carcinoma, and the vaccine can thus be considered the first anticancer vaccine ***(3).*** Ideally, the antibody response (anti-HBs antibody level) is determined within one to three months after the last dose of the vaccine in persons with risk factors for lack of response or those at high risk for exposure to blood or bodily fluids. Unfortunately, the antibody response is frequently tested years after completion of vaccination series, in which case a true non-response (an antibody level of < 10 mIU/mL after appropriate vaccine series) must be distinguished from ″ waning″ antibody levels (those levels of antibody that are initially protective but that become undetectable over time). Administration of a single dose of vaccine, followed by measurement of the anti-HBs antibody response 4 to 12 weeks later, will differentiate persons with no response (indicating an absence of protection against the disease) from those with waning antibody levels (in whom an anamnestic response will occur, with anti-HBs antibody levels of at least 10 mIU/ mL) ***(4).***

Few data are available concerning the long term immunogenicity of the pediatric doses of hepatitis B vaccines but the immunity persists for at least 5 years after the primary vaccination ***(5).*** ***Van der Sande et al.*** stated that HBV vaccination early during life can provide long-lasting protection against carriage, despite decreasing antibody levels. The role played by subclinical boosting and the necessity of a booster need to be evaluated (6), While ***Puvacic et al.*** cleared that vaccination against viral hepatitis B results in immunologic memory response among the vaccinated and even after a decrease of anti-HB level following the third vaccine dose inoculation, a booster dose is not needed. Immunity remains steady and a booster dose is not recommended. The aim of the work is to determine the level of HBsAb in children who were primarily vaccinated by the 3 doses of HBV vaccine after five and ten years of vaccination in order to detect the long-term immunogenicity of the vaccine, also to test for anamnestic reaction in those with declining levels of antibody to determine whether or not a booster dose is needed (7).

METHODS

This study included 200 healthy children. The children were divided into two groups according to age (each group contains 100 children). Their data are included. Group `A` were around 6 years old, this group included 53 males and 47 females. Group `B` were around 11 years old, this group included 27 males and 73 females).

***Selection criteria of cases*:** Normal children with no liver diseases or any other chronic illness and not on long term steroids, as not to be

immunocompromized, detected by history taking and clinical examination. A questionare filled in with the parents was prepared and fulfilled for each child included in this study to check for the history of liver disease and any other diseases.

Included children must be free of HBV infection, so screening for HBV in the children's blood was done by testing for HBsAg, HBcIgG, HBcIgM.

All children should have received their three doses of HBV vaccine at 2, 4 and 6 months according to the EPI in Egypt

Normal growth and nutritional status, this was detected by taking the anthropometric measures for each child (weight, height and mid-arm circumference), also serum albumin level was tested and only normal children were included.

***All children were subjected to*:**

1. History taking, 2. Clinical examination, 3. Investigations

Anthropometric measures were taken for each child, Anthropometric measurements are simple, safe and inexpensive. The measurement of growth parameters in a child is one of the most sensitive and commonly used indicators of child health ***(8)*** . Height and weight measurements are the mainstay of the nutritional assessment of the child ***(9).*** Body weight is commonly used as an indicator of general nutrition status. Length/ Height are affected rather late in malnutrition; therefore, the short stature of children may represent growth failure due to chronic under nutrition ***(10)***. Measurement of mid-arm circumference (MAC) is also useful in detecting malnutrition. This is a measure of muscle mass, which is reduced in all types of malnutrition. MAC is a simple, inexpensive and practical screening tool for assessment of malnutrition ***(11).*** In this work we used the Z-score included in the international growth references, produced by the National Center for Health Statistics (NCHS), Center for Disease Control and Prevention, and WHO.

***HBsAb Assay :Principle of Anti-HBs analysis*:** Anti-HBs is a specific (generally IgG) antibody that is directed against the hepatitis B surface antigen. Anti-HBs can be formed following a hepatitis B infection or after vaccination. Antibodies are formed against the HBsAg determinant `a`, which is common to all subtypes, and against

subtype-specific determinants. The Elecsys Anti-HBs assay uses a mixture of purified antigens of the HBsAg subtypes `ad` and `ay` from human serum

***Sandwich principle*:**(Total duration of assay is 18 minutes):-

- *First incubation:* Anti-HBs in sample (40ul), biotinlyted HBsAg (ad/ay) and HBsAg (ad/ay) labeled with a ruthenium complex react to form a sandwich complex.
- *Second incubation*: after addition of streptavidin-coated micropaticles the complex become bound to solid phase via interaction of biotin and streptvidin.
- The reaction mixture is aspirated into measuring cell where the micropaticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Procell solution. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by photomutiplier.
- Results are determined via a calibration curve which is an instrument specifically generated by 2-points calibration and a master curve provided via the reagent barcode.

Anti-HBs persistence was defined as an anti-HBs concentration of ≥ 10mIU/ mL ***(12)*** Children whose antibody titre

was < 10 were given an extra dose of HBV vaccine [Hepatitis-B Vaccine, B.P (r-DNA), genetically engineered recombinant vaccine, VACSERA] manufactured by VACSERA under license of SHANTHA BIOTECHNIC. Pediatric dose = 0.5 ml IM, one ml contains 20µg HBsAg (purified). This dose is given to test for anamnestic reaction and HBsAb was measured again after one and half month. Successful response to vaccination was defined as anti-HBs ≥ 10 mIU/ mL from 0.5 to 20 months after vaccination ***(12).***

RESULTS:

Regarding growth and nutritional status, children in group (A) were as follows: males` weight range of 21-34 kg with mean value of 27.24 ± 3.31, the mean Z score for weight of boys was 1.4± 0.9, their height ranges from 121 to 139 cm with mean of 129± 4.44, Z score was 2± 0.85, the measurements of mid-arm circumference were between 14–23cm with mean value of 17.45 ± 1.8 and albumin level was from 3.8– 5.6g/dl with mean of 4.49 ± 0.32. As for females, their weight ranges from 22–35 kg with mean of 27.76 ± 3.12, Z score mean was 1.5± 0.7. Height lies between 122 and 139 cm with mean = 128.59 ± 4.48 and mean of Z score was 1.8± 0.8, mid-arm circumference ranges from 15 – 22 cm with mean = 17.95± 1.62 and albumin level was between 3.8 and 5.9g/dl with mean = 4.5 ± 0.4.

As for group B, the data were as follows: weight of males range of 27-71 kg with mean value of 36.44 ± 8.5, the mean Z score was −0.3 ± 1.1, their height was 126- 149 cm with mean = 139.40± 5.08 and Z score mean was −0.9± 0.7, mid-arm circumference was 17 - 32 with mean of 21± 3.02 and the albumin ranges from 3.9 to 5.5, its mean = 5± 0.39. Females` data were as follows: weight range is 25 – 66 kg with mean = 39.78 ± 10.27, with mean Z score of − 0.1± 1.2, their height was 129 - 160, the mean was 143.16± 7.92 and the mean of Z score was −0.5± 1, mid-arm circumference was 14 - 32 with mean = 21.31± 3.05 and lastly albumin was 4.1 - 6 with mean of 5.10 ±0.38.

When testing the serum of the children in both groups (A&B) for HBsAb and its titre there was significant difference between the two age groups. While the median level of HBsAb in group A was 36.22, it was 9.8 in group B. This difference has significant p value = 0.000. Both groups have a wide range concerning the level of anti-HBs (2→ 1000) mIU/ml. This data is a proof for the decline of the level of antibodies with time. When comparing the HBsAb titre between girls and boys in each group, there was no significant difference between girls and boys in group A but in group B, there was significant increase in the median level of HBsAb in boys than girls. The median of HBsAb for males in group A was 37.43, as for females, it was 35.02 with p value = 0.462 which is not significant. The median of HBsAb for males in group B was 14.88 compared to females which was 6.63, the p value = 0.000 which is significant. This is shown in **Table 1, 2,3.**

“““““Levels of HBsAb ≥ 10mIU/mL are universally considered to be protective against HBV infection. So children whose results are ≥ 10 are protected while those having levels < 10 are not protected initially post vaccination and considered to be non-responders, but children tested years after being

vaccinated may show a decline in the level of antibodies while retaining immunologic memory, therefore children in this condition whose antibody titre was found to be < 10 must be tested for anamnestic reaction by an additional dose of the vaccine. In this study when testing HBsAb of children in group A, children having titre ≥ 10 were 81 child (44 males i.e. 83% of males & 37 females i.e. 78% of females) and those having titre <10 were 19 child (9 males i.e. 17% of males & 10 females i.e. 22% of females) with no significant difference between boys and girls as p value was 0.585. Those 19 children were in need of an additional testing dose of the vaccine to search for their anamnestic reaction. In group B: 52 children had titre < 10 (6 males i.e. 22.2% of males and 46 i.e. 63% of females), their immune response was in need to be tested. Forty eight children had titre of ≥ 10 (21males i.e. 77.8% of males, 27 females i.e. 37% of females). So there is significant difference between boys and girls in group B with p value = 0.000.

Table 1: Comparison between group A and group B regarding level of HBsAb

test		Group A	Group B	p
HBsAb mIU/mL	**Range**	**2-1000**	**2-1000**	**0.000**
	median	**36.22**	**9.8**	

Table 2: Distribution of children in group A and B according to HBsAb titre.

HBsAb titre	Group A	Group B	P
< 10 mIU/ mL	19 (19%)	52 (52%)	0.000*
≥ 10mIU/ mL	81 (81%)	48 (48%)	

Table 3: Comparison between males and females in each group regarding level of HBsAb.

Age group		Male	Female	P
Group A	Range Median	(4.2- 1000) 37.43	(2.2- 1000) 35.02	0.462
Group B	Range Median	(3.3- 1000) 14.88	(2.0- 99) 6.63	0.000*

On comparing children having HBsAb titre of < 10 and those with ≥10 as regards their nutritional status and growth using indicators of growth and nutrition; weight, height, MAC and albumin, there was no significant difference in any of these parameters in each sex in the two groups. So, growth and nutritional status can be excluded as a cause of decreased HBsAb level.

In group A, from the nineteen children who needed an additional vaccination dose 14 were vaccinated (4 children were lost & the parents of one child refused to continue), after one and half month from vaccination another sample was taken from 10 children; (Three children were lost & the mother of one child refused to continue after receiving the vaccine). Out of these 10, 9(90%) respond by increased level of HBsAb and only one child did not responded. Six (66.6%) of the nine showed an adequate response i.e. HBsAb ≥ 100, three were hyporesponders i.e. HBsAb ranges from 10-100. As for group B there was no significant difference in responding to the testing dose with p value =0.814 which is not significant. Fifty two children in this group had antibody titre < 10, forty eight were vaccinated (Three children were lost and the father of one child refused the vaccination). After one and half month 34 children (5 males and 29 females) were tested again for HBsAb (14children were lost after receiving the vaccination). Two out of the thirty four children don`t respond (5.8%) and 32 (94.2%) responded by an increase in the antibody titre. Of those responded 19 (4males and 15 female) had adequate response (HBsAb ≥ 100) and 13 (a male and 12 female) had hypo-response (HBsAb lies between 10-100). Therefore, 80% of the boys who were retested for HBsAb after vaccination respond adequately while 51.7% of the corresponding girls respond adequately. **Table 4** presents the comparison between children in group A and B in their response to the additional vaccination dose also a comparison between boys & girls in the response.

Table 4: Comparison between groups A & B: level of HBsAb after booster dose of vaccination.

Test		Group A (n=10)	Group B (n=34)	P
HBsAb after vaccination mIU/ mL	**Range** Median	(8.9-1000) 579.65	(3.23-1000) 230.95	0.887

Table 5: Comparison between males and females: level of HBsAb after vaccination dose

	Sex	HBsAb after vaccination	p
Group A	**M (n=3)**	(65.42-1000) 588.6	0.648
	F (n=7)	(8.9-1000) 570.70	
Group B	**M (n=5)**	(18-1000) 1000	0.311
	F (n=29)	(3.23-1000) 144.6	

DISCUSSION

HBV infection is preventable with safe and effective vaccines that have been available since 1982. The vaccine is 95% effective in preventing chronic infections from developing, and is the first vaccine against a major human

cancer. More than 160 countries have already added this vaccine to their routine immunization programmes ***(13).*** In 1992, Egypt started a program of universal immunization in infancy. The schedule adopted by Egyptian Ministry of Health was three doses of yeast-recombinant hepatitis B vaccine administered to all infants at 2, 4, 6 to coincide with other compulsory vaccines (Diphtheria, Tetanus, Pertussis and oral polio (DPT- OPV) ***(14). Van der Sande et al.*** stated that carriage of hepatitis B virus (HBV) is a major risk factor for liver cirrhosis and hepatocellular carcinoma. Infant vaccination has been effective in preventing horizontal transmission during early childhood. It is unknown whether protection is maintained into early adulthood. In their study, they concluded that HBV vaccination early during life can provide long-lasting protection against carriage, despite decreasing antibody levels A better understanding of protective immune responses (correlates of protection) and immunologic memory are required to facilitate the improvement of vaccination programs (***15).***

‘‘‘‘‘‘This study included 200 healthy children, divided into two groups according to time elapsed since they had received their initial vaccination. Group (A) included children who were vaccinated 5 years ago while group (B) included those vaccinated 10years ago (at the time samples were drawn). HBsAb titre was tested in their blood, an additional dose of the vaccine was given to children whose HBsAb was < 10 mIU/ml then one and half months later another blood sample from each of them was retested for HBsAb to evaluate the response to this dose of the vaccine. Before being included in the study children were screened for the presence of HBV infection.ALT, HBsAg, HBcIgM, HBcIgG were tested, none of them had abnormal liver function or positive serologic markers for HBV infection. This is a proof for the efficacy of the vaccine. The results of our study show statistically significant difference between group A and B in levels of anti-HBs with p value = 0.000 and this shows that the level of antibody against HBV declines with time. This is in concordance with other investigators. ***Dentinger et al.*** conducted a study to evaluate the long-term protection of hepatitis B vaccination among children immunized when infants and found that anti-HBs concentration dropped rapidly among all participants. Five years after vaccination of children who had received recombinant vaccine, 6% only had HBsAb ≥10mIU/ml and 3% of them at 10 years retain the protective titre (12). In our study 81% and 48% retain immune protective levels of ≥ 10mIU/ ml after 5 and 10 years respectively These differences may be attributed to the more frequent inapparent exposures to HBV and boosts in anti-HBs in the community of children included in our study, as they performed their study on a less endemic community.

In our study when comparing growth and nutritional status of children with HBsAb <10mIU/ ml regarding parameters as height, weight, MAC and albumin with those having titre ≥ 10 and also by assessing growth of children who didn't respond to the additional dose of the vaccine, the results showed no statistically significant difference. On the contrary to that ***Keating et al***. reported that the greater the body mass index the less the immune response (16), while ***Charles et al***. showed that advancing age, obesity and smoking in adults have negative influence on the efficacy on hepatitis B vaccination and explained that effect by the deposition

of the vaccine in fat rather than in muscle resulting in higher failure rates (17). Also***, it was*** reported that many host and immunization factors affect the immune response and consequently can influence the duration of immunity. Host factors include age, weight, immunocompetence of the host, smoking habits, body mass index and genetics Some investigators correlates the socioeconomic state with vaccine response (18)***. Wang et al.*** reported that universal hepatitis B vaccination program (UHBVP) was less effective in socio-economically disadvantaged area and the long-term efficacy and immunogenicity of vaccination were modified by host factors and factors associated with urbanization. They correlate this with levels of HBsAb but none of these indicators show significance. When testing the presence of immunologic memory by an additional dose of the vaccine, most of children in our study show positive anamnestic reaction with increase of HBsAb but with no statistically significant difference between the two groups A and B as p value was 0.887. Children who didn't respond to the testing dose of vaccination are either primary non-responders to the initial vaccination or may have lost their immunologic memory. So these results prove that children who were successfully vaccinated at birth are not in need for a booster dose of the vaccine at least for 10 years. This was in agreement with some investigators (19).

Puvacic et al. reported that after five years of vaccination, long term immunogenicity of vaccinated children remained at 88.89% (81% in our study) and that vaccination against viral hepatitis B results in immunologic memory response among the vaccinated, and even after a decrease of anti-HB level following the third vaccine dose inoculation, a booster dose is not needed. Immunity remains steady and a booster dose is not recommended (7). Also, ***Sjogren*** stated that the distinction between true nonresponse (after adequate immunization) and waning anti-HBs levels is important. The latter is not uncommon in populations in areas of the world with low endemicity for HBV infection (20). Data from subjects with waning anti-HBs levels show that immunologic memory may still protect these individuals against acute HBV infection or may prevent chronic infection with HBV for < or =10 years after immunization. In a long study for 18 years, ***Yuen et al.*** stated that the long-term immunogenicity and efficacy of hepatitis B virus (HBV) vaccination remain to be defined. They aimed to examine the long-term immunogenicity and efficacy of HBV vaccination over 18 years of follow-up and concluded that because of the highly effective anamnestic responses, a booster dose was not necessary at least up to 18 years after the primary vaccination (21).

Dentinger et al. reported that up to age 16 years, booster doses of HB vaccine are not required to protect against clinically significant disease. The results of a small study revealed that approximately one-fourth of successfully vaccinated infants with anti- HBs < 10mIU/ml by early adolescence fail to mount an anamnestic response to a booster dose of HB vaccine Whether booster doses of vaccine are needed for long term protection into late adolescence and adulthood need to be clarified (12). Other investigators still raise the possibility for a booster dose , ***Wang et al.*** cleared that their previous study suggests that routine booster vaccination may not be necessary to

provide protection against chronic HBV infection before age 15 years, as the maintenance of HBsAg-specific memory confers protection against a clinical breakthrough infection even in the absence of detectable antibodies. However, the possibility of a need for a booster dose exists, particularly when the child becomes adolescent. Whether primary HBV vaccination in infancy can provide protection in adolescence remains to be elucidated (22).

Also, ***El-Sawy and Mohamed*** evaluated the long-term immunogenicity and efficacy of HBV vaccination in children whose time lapse since last vaccination varied between 1 month and 5 years. They found that there were low initial anti-HBs concentrations and it declined rapidly over time. They recommend booster inoculations for all previously vaccinated children and a new vaccination schedule at 1, 2 and 9 months. Lastly this study detected that males may retain anti-HBsAb titres of higher values than females. There was statistically significant difference in levels of HBsAb between boys and girls in group B. There was less number of boys with anti-HBs < 10 mIU/ ml (22.2% of males included in group B ≠ 63% of females of the same group) (23). Also boys who received the additional vaccine dose responded more adequately than girls (level of HBsAb was ≥ 100mIU/mL) This agrees in part with some investigators. ***McMahon et al.*** stated that initial anti-HBs level, older age at vaccination and male sex were associated with persistence of higher anti-HBs levels at 15 years (24). But ***Elsawy and Mohamed*** showed that there was no statistical difference between boys and girl ***(23).***

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REFERENCES:

1. **Lok AS, Heathcote EJ and Hoofnagle JH.** Management of hepatitis B: 2000-summary of workshop. Gastroenterology **2001:** 120 (7): 1828-53.
2. **Yu AS, Cheung RC and Keeffe EB.** Hepatitis B vaccines. Clin Liver Dis **. 2004:** 8(2): 283-300.
3. **Zuckerman AJ.** Prevention of primary liver cancer by immunization. N Engl J Med**. 1997:** 336: 1906-1907.
4. **Gregory A Poland and Robert MJ.** Prevention of hepatitis B with hepatitis B vaccine. N Engl J Med **. 2005:** 352(7): 7410.
5. **Duval B, Gilca V, Boulianne N, De Wals P, Masse R, Trudean G and de Serres G.** Comparative Long Term immunogenicity of two recombinant hepatitis B vaccines and the effect of a booster dosegiven after five years in a low endemicity country. Pediatr Infect Dis J**. 2005:** 24(3): 213-218.
6. **Van der Sande MA, Waight P, Mendy M, Rayco-Solon P, Hutt P, Fulford T, Doherty C, McConkey SJ, Jeffries D, Hall AJand Whittle HC.** Long-term protection against carriage of hepatitis B virus after infant vaccination. J Infect Dis. **2006:** 193(11):1528-35.
7. **Puvacic S, Ravlija J, Puvacic Z and Curic I.** Long term protection after hepatitis B

vaccination. Bosn J Basic Med Sci. **2005:** 5(3):50-3.

8. ***World Health Organization.*** Growth monitoring of preschool children. Practical consideration for the PHC projects. Primary Health Care Issues. ***1981:*** 1(3): 1-70.
9. ***Motil KJ.*** Sensitive measures of nutritional status in children in hospital and in the field. Int J Cancer Suppl. ***1998:*** 11:2-9.
10. ***Harris N, Yangzom Y, Pinzo I, Gyaltsen P and Hudes M.*** Nutritional and Health Status of Tibetan Children Living at High Altitudes. N Engl J Med***. 2001:*** 344 (5):341-347.
11. ***Berkley J, Mwangi I, Griffiths K, Ahmed I, Mithwani S, English M, Newton C and Maitland K.*** Assessment of severe malnutrition among hospitalized children in rural Kenya: comparison of weight for height and mid upper arm circumference. JAMA***. (2005:*** 294(5):591-7.
12. ***Dentinger CM, McMahon BJ, Butler JC, Dunaway CE, Zanis CL, Bulkow LR, Bruden DL, Nainan OV***, ***Khristova ML, Hennessy TW , and Parkinson AJ.*** Persistence of antibody to hepatitis B and protection from disease among Alaska natives immunized at birth. Pediatr Infect Dis J. ***2005:*** 24(9):786-92.
13. **Kiran V.** Hepatitis-B vaccine introduction into the routine immunization schedule--Andhra Pradesh experience. Indian J Public Health. **2004:** 48(2):63-6.
14. ***Mansour E, Abdul-Rahim S, Batouty G, Zaghloul I and Abdel-Hadi S (1993):*** Integration of hepatitis B immunization in the Expanded Program on Immunization of the Child Survival Project. J Egypt Public Health Assoc; 68(5-6):487-94.
15. **Van der Zeijst BA, Dijkman MI, Luytjes W, Van Alphen AJ and van den Dobbelsteen GP.** On the design of national vaccination programmes. Vaccine. **2007:** 25(16): 3143-5.
16. **Keating, Gillian M, Noble et al. R**ecombinant hepatitis B vaccine (Engerix-B): A review of its immunogenicity and protective efficacy against hepatitis B. Adis international drugs. **2003:** 63(10): 1021-1051.
17. **Charles J, Leah Kelly et al.** Hepatitis B vaccination in pregnancy, factors influencing efficacy. J Obst and Gyna. **1999:** 93(6): 983-989.
18. **Tseqa E and Lin.** Antibody levels in Ethiopian children five years after vaccination with two different doses of hepatitis B vaccine: is there a need for booster vaccination? Canadian J Gastroenterology. **1998:** 12(1): 57-60.
19. **Wang LY, Hu CT, Ho TY and Lin HH.** Geographic and ethnic variations of long-term efficacy and immunogenicity of hepatitis B vaccination in Hualien, a HBV hyperendemic area. Vaccine. **2006:** 24(20):4427-32.
20. **Sjogren MH.** Prevention of hepatitis B in non-responders to initial hepatitis B virus vaccination. Am J Med**. 2005:** 118 Suppl 10A:34S-39S.
21. **Yuen MF, Lim WL, Chan AO, Wong DK, Sum SS and Lai CL.** 18-year follow-up study of a prospective randomized trial of hepatitis B vaccinations without booster doses in children. Clin

Gastroenterol Hepatol. **2004:** 2(10):941-5.

22. **Wang JS, Chen H and Zhu Q.** Transformation of hepatitis B serologic markers in babies born to hepatitis B surface antigen positive mothers. World J Gastroenterol**. 2005:** 11(23):3582-3585.
23. **El-Sawy IH and Mohamed ON.** Long term immunogenicity and efficacy of recombinant hepatitis B vaccine in Egyptian children. J. Eastern Mediterranean health**. 1999:** 5(5): 922-932.
24. **McMahon BJ, Bruden DL, Petersen KM, Bulkow LR, Parkinson AJ, Nainan O et al.** Antibody levels and protection after hepatitis B vaccination: results of a 15-year follow-up. Ann **Intern Med. 2005: 142(5):333-41.**