COMPARING HEPATITIS B SEROPROTECTION IN PAKISTANI CHILDREN AT 5 AND 6 YEARS OF AGE AFTER EXPANDED PROGRAMME ON IMMUNIZATION (EPI) VACCINATION

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ABSTRACT

Objective: To compare seroprotection of children at 5 and 6 year of age after receiving Hepatitis B vaccination according to Expanded Programme on Immunization (EPI).

Study Design: Cross sectional study.

Place and Duration of Study: The study was conducted in the Department of Paediatric Medicine, Pak Emirates Military Hospital, Rawalpindi, from Oct 2015 to Apr 2016.

Methodology: After the approval of ethics committee, informed consent of participants was obtained. Total 85 children, both male and female, aging 5 and 6 years were included in study fulfilling inclusion criteria. 3.5 ml blood samples were obtained ensuring antiseptic precautions using disposable syringes. Samples were taken in vaccutainers, labeled with patient's demographic information and were immediately transported to the Armed Forces Institute of Pathology (AFIP) for analysis. Hepatitis B surface antibody titers of >10 IU/ L was taken as protective and <10 IU/L were considered negative. Children with anti-HBs titer <10 IU/L were sent for revaccination. Data was analyzed using SPSS version 22.

Results: Out of 85 children, 41 (48.2%) were female and 44 (51.8%) male. Mean anti hepatitis B antibodies levels was 92.15 ± 66.03 IU/L. There were 32 (74.4%) seroprotected children who have age of five years and there were 20 (47.6%) seroprotected children who were six years old.

Conclusion: Our study concluded that with increasing age, significant number of children have decline in seroprotection against hepatitis B after receiving vaccination according to EPI schedule.

Keywords: Anti-HBs antibodies, EPI, Hepatitis B vaccination, Seroprotection.

INTRODUCTION

Hepatitis B virus (HBV) causes acute and chronic liver disease and has an average incubation period of 120 days[1]. It's modes of transmission include vertical transmission like perinatal and horizontal transmission like sexual contact, unsafe injection practices and infected blood transfusions[2]. The prevalence of HBV varies greatly in different parts of the world. According to World Health Organization (WHO) reports, more than two billion individuals are infected with HBV around the world. In Pakistan, research carried out at different setups shows a prevalence of 3-4%, which makes Pakistan an area of intermediate endemicity for Hepatitis B[2]. In 2015 a research was done in Rawalpindi which shows that 5% of adults, 20% of young and 90% of neonates infected with HBV leads to chronic hepatitis. Infection in early life is a risk factor for development of chronic hepatitis, cirrhosis and hepatocellular carcinoma in later life[3].

The most effective infection control in a country with Hepatitis B endemicity like ours is effective vaccination which saves both lives and money and is also recommended by WHO[3]. Due to effective vaccination of children, a dramatic decrease in the prevalence of hepatitis B has been observed[4]. In 2002, global alliance for vaccination and immunization (GAVI), allotted grant to

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Pakistan. The grant enabled Pakistan to include Hepatitis B vaccine in EPI schedule[5].

HBV DNA is the first viral marker which appears after infection with HBV, after which Hepatitis B surface antigen and Hepatitis B e-antigen appears and can be detected in serum of patient. Hepatitis B surface antigen can be found within first weeks, but sometimes it cannot be traced until 11–12 weeks following infection. Persistence of Hepatitis B surface antigen in patient serum denotes chronic infection. HBV replication and infectivity can be related to increase level of Hepatitis B e-antigen[6]. After the appearance of serum markers, serum alanine transaminase and Aspartate transaminase (aminotransferase) become elevated and by this time patients are usually icteric. Generally, Hepatitis B e-antigen disappears early from patient blood. On the other hand HBV DNA and HBsAg often continue to detect throughout illness. HB antibodies appear invaried patterns during different stages of infection, so knowledge of these patterns helps to make accurate diagnosis about stage of disease. Disappearance of Hepatitis B e-antigen and presence of anti-HBe is anappropriate marker during acute stage of hepatitis B; this also shows the beginning of recovery. Anti-HBs appears quite late, usually with recovery from infection and after disappearance of HB surface antigen. Anti-HBs is associated with immunity against HBV because it lasts long after recovery. About 10% and 15% of patients who suffer from hepatitis B infection do not have measurable anti-HBs, so anti-HBc alone serves as aindicator of past infection. That is why, best and consistent marker of evaluating previous infection with HBV is anti-HBc, whereas most reliable method of evaluating immunity after HBV vaccine is anti-HBs[7].

Anti-HBs Surface antibody titers tend to decline with time after vaccination[5]. Time since vaccination is the strongest risk factors for Hepatitis B infections in immunized children, therefore some studies recommend a booster dose of vaccination due to increased chances of developing new HBV infections after falling Anti HBs titers[8]. After going through various studies certain grey areas can be identified. Firstly, considering the sensitivity and magnitude of Hepatitis B in our set-up, proportionate work is not done to evaluate seroprotection after EPI vaccination. Secondly various studies conducted in different parts of the world to assess seroprotection show variable results. Thirdly study conducted in our country shows low protection level as compared to international data which further necessitate research on this subject.

As Hepatitis B is endemic in Pakistan and vaccine-induced antibody titers to Hepatitis B surface antigen (i.e. Anti-HBs) also reduce with time, the rationale of our study was to evaluate seroprotection level in our population at 5 years and 6 years of age after routine Hepatitis B vaccination according to EPI.

METHODOLOGY

This was a cross-section study conducted Paediatric Medicine, Department at Military Hospital Rawalpindi from October 2015 to April 2016. Sampling technique used was consecutive non-probability sampling. Sample size was (n) was 85 patients considering our inclusion criteria, which was calculated using WHO sample size calculator taking confidence level=95%, anticipated population proportion = 68.6% and absolute precision required = 10%. Both male and female children aging 5 and 6 years were included in study who had received 3 doses of Hepatitis B vaccine at 6, 10 and 14 weeks as per EPI schedule. We also ensured that children included in our study must have a complete record of vaccination properly endorsed in their vaccination cards and they have completed 5 or 6 years since vaccination. We excluded those children from our study who were having acute illness at time of sampling. Children on immunosuppressive medications, having history of blood transfusion or suffering from chronic illness like chronic renal failure/chronic liver disease were not included in our study. Hepatitis B surface antibody titers of >10 IU/L was taken as protective against HBV infection and levels <10

IU/Lwere considered negative. Children who were found to have anti-HBs titer <10 IU/L were sent back for re vaccination. The following operational definitions were used.

Hepatitis B Surface Antibody: Hepatitis B surface antibody were measured by ELISA (Enzyme linked immunosorbent assay) in IU/ L at Armed Forces Institute of Pathology (AFIP).

Seroprotected: According to WHO standards, Hepatitis B surface antibody titers of >10 IU/ L was taken as protective against HBV infection.

Approval of hospital ethics committee was sought for study. We obtained informed consent from parents or guardians of all participants of our study before blood sampling. We also conveyed results of Hepatitis B Surface antibody screening to all participants of the study. Proper antiseptic precautions were ensured. After which blood samples (3.5 ml) were obtained by venipuncture using disposable syringes. Blood samples were collected in vaccutainers and labeled with patient's demographic information. Samples were immediatelly transported to the Armed Forces Institute of Pathology (AFIP), where these were retained straight for 30 minutes. For 10 minutes samples were centrifuged, supernatant serum was collected and preserved at -20 degree centigrade until test was done. Testing was done using Enzyme linked immunosorbent assay (ELISA) kit as per manufacturer's guidelines. SPSS version 22 was used for analysis of data. Quantitative variables like age and Anti HBs antibodies levels were calculated by mean and standard deviation. Qualitative variables like gender and seroprotection in children were calculated in terms of frequency and percentages. Effect modifier like age and gender were stratified. Chi-square test was used for post stratification keeping *p*-value ≤0.05 as significant.

RESULTS

Eighty five children fulfilling inclusion criteria were included in the study. In our study mean age (years) was 5.49 ± 0.50. Gender distribution of patient was considered in terms of male and female patients. In this study there were 44 (51.8%) male and 41 (48.2%) female patients. Mean of anti hepatitis B surface antibody levels was 92.15 ± 66.03 IU/L. Effect modifier like gender stratification was compared with those children who are seroprotected after receiving hepatitis B vaccination. There were 26 (59.1%) male and 26 (63.4%) female children who were seroprotected after receiving hepatitis B vaccination. Effect modifier like age stratification was compared with those children who are seroprotected after receiving hepatitis B vaccination. There were 32 (74.4%) seroprotected children who have age of 05 years and there were 20 (47.6%) who have 06 years of age. Chi-square test was used to compare age stratification with seroprotection after receiving hepatitis B vaccination which was statistically significant (*p*-value 0.011), as shown in table-I. Chi-square test was used to compare gender stratification with seroprotection after receiving hepatitis B vaccination which turned to be statistically insignificant (*p*-value 0.683), as shown in table-II.

Table-I: Association of age groups with Seroprotection.

		Seroprotection		*p*-value
		Yes (%)	No (%)	
Age group	5 years	32 (74.4)	11 (25.6)	0.011
	6 years	20 (47.6)	22 (52.4)	

Table-II: Association of gender with seroprotection.

		Seroprotection		*p*-value
		Yes (%)	No (%)	
Gender	Male	26 (59.1)	18 (40.9)	0.683
	Female	26 (63.4)	15 (36.6)	

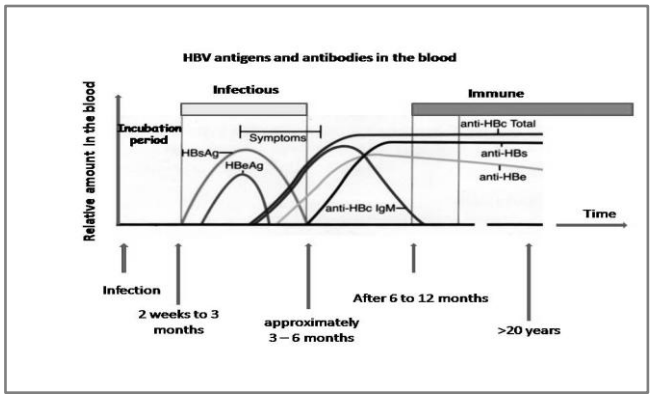

Figure: Serum markers in blood after HBV infection.

DISCUSSION

Hepatitis B is a major public health issue with variable incidence throughout the world. HBV infection is mostly without symptoms but it has long term problems which occur after many years. The most important factor is age at which child acquires infection and it in turn decides acute or chronic nature HBV disease. If a mother who has both HB surface antigen (HBsAg) and HB e antigen (HBeAg) positive, gives birth to a child, the risk is 70% - 90% that baby will have chronic HBV infection by the age of 6 months if no post-exposure immunoprophylaxis is administered[9,10]. In 2015 a research was done in Rawalpindi which shows 5% of adults, 20% of young and 90% of neonates infected with HBV leads to chronic hepatitis. Research carried out in various cities of Pakistan show prevalence of 3-4%, which makes Pakistan an area of intermediate endemicity for Hepatitis B. In a country with high prevalence of HBV, the population has manifold increased risk of getting infected with HBV infection[11].

Hepatitus B vaccine can truly be called as the first anti-cancer vaccine since it prevents development of liver cancer. Hepatitis B Vaccine is the only hopeful method for prevention of HBV infection and its complications. After discovery of HB vaccination, detailed policy to eradicate HBV transmission was deliberated internationally[9]. Primary HB vaccination is administered as 3 dose series to children by intramuscular route. Hepatitis B vaccine is delivered by government health infrastructure to hospitals and other medical setups, where it is administered. Research shows protection rates of 95% after immunization of infants and children at 0, 1 and 6 months[12]. This figure decline with increasing age[13].

Anti-HBs antibodies are used to measure development of immunity after Hepatits B vaccination. An anti body levels of 10 IU/L more than is taken as protective. About 5–15% of children do not develop protective level of this antibody. Multiple factors effect development of antibodies after HB vaccination and that's the reason of diverse seroprotection results. Vaccination dose, dosing schedules, route and site of administration, storage, age and gender of recipient affect HB antibodies development after vaccination[12]. Serum markers appearing in blood after HBV infection are shown in figure.

Going through available literature few short comings can be identified in our HB vaccination schedule. First is the dosing schedule that has pivotal role in the antibody response and Anti HBs titres. American Advisory Committee on Immunization Practices recommends a 8 weeks gap between 2nd and 3rd doses of HB vaccination and 1st and 3rd dose should be administered at least 16 weeks apart[14]. In order to facilitate our population and to improve adherence to vaccination regime, the dosing schedule has been adjusted at 6, 10 and 14 weeks in our EPI schedule along with other vaccinations. Second observation on EPI regime regarding Hepatitis B Vaccination is that it does not have very important birth dose which is 70-95% protective even if administered without HB immunoglobulins. These factors may be the cause of less seroprotection in our population[15].

Results of our study are different from published literature. Afzal *et al*[12] in their study found that the seroprotection was low in female gender with a significant difference without any plausible explanation. Whereas no such difference was found in our study and seroprotection was 26 (59.1%) male and 26 (63.4%) among female children after receiving hepatitis B vaccination. In our study, there were 32 (74.45%) seroprotected children who have age of 05 years and there were 20 (47.6%) seroprotected children who were 6 years of age. In our study, mean anti HBs antibodies levels was 92.15 ± 66.03 IU/L whereas in a study by Aghakhani *et al*[16] the mean level of anti-HBs was 66 ± 38 mIU/mL.

Our study revealed that there were 32 (74.4%) seroprotected children who have age of 5 years and there were 20 (47.6%) seroprotected children who were 6 years of age. This seroprotection level is less when compared to interna-

tional data[13,17]. The difference may be due to the different vaccination schedule in these countries. This problem is further magnified when we realize that coverage rate of vaccination is also low in our country. Resultantly high proportion of our children are at risk of acquiring HBV infection even after EPI schedule and eradication of this preventable disease remains a dream. In another African study, where children were vaccinated at 6, 10 and 14 weeks (same as our schedule), revealed waning seroprotection with increasing age[18]. We have made an attempt to highlight actual scenario of seroprotection after EPI vaccination which will help in formulating new strategies and vaccination schedule. This will definitely be a step toward eradication of HBV infection and liver diseases from our country.

CONCLUSION

Our study showed that with increasing age, significant number of children have waning immunity against hepatitis B, after receiving vaccination according to EPI schedule. There is a need to improve seroprotection by rectifying the factors likevaccination dose, type, dosing schedules, site and route of vaccination, monitoring and training of vaccination teams and storage methods to ensure cold chain maintenance, since all these influence development of HB antibodies. This will help achieving desired seroprotectionrate which willguard our children against this preventable infectious disease.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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