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Hepatitis B Seroconversion after Vaccination in Infants in Rural and Urban Areas of Rawalpindi, Pakistan

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Authors' contributions

This work was carried out in collaboration between all authors. Author IP designed the study with assistance of authors SA and SSZZ wrote the protocol and conducted all the experimental work as well as wrote the first draft of the manuscript. Authors SS and MAR managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Purpose: This study was designed to evaluate the immunogenicity of hepatitis B vaccine in infants of rural and urban Pakistan and to find out the vaccine efficacy.
Methods: A sample of 400 subjects was taken by simple random method from the rural and urban areas (200 from Rural and 200 from urban areas) of Rawalpindi District Pakistan. The serum samples were analyzed by Enzyme Linked Immunosorbant assay (ELISA) for the quantitative determination of antiHBs antibodies.
Results: As 88 (22%) infants out of the total 400 were found to be seropositive at baseline. The post vaccination results showed that about 15 (7.53%) of infants had inadequate levels of antibodies (i.e. <10 IU/L). Overall 92.46% infants showed a positive response to the vaccine. The females showed higher titers of antiHBs against vaccination as compared to that of males. Infants

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of urban areas showed higher titers as compared to that of rural areas.
Conclusion: Our results reinforces that the Hepatitis B vaccine has a good tolerability and is highly immunogenic among infants. It is recommended that serosurvey of HBsAg and vaccine coverage at country level should be done.

Keywords: Enzyme-linked immunosorbant assay; immunogenicity; hepatitis B; hepatitis B vaccine engerix (TM)-B.

1. INTRODUCTION

Chronic infection with HBV can lead to severe medical outcomes, including cirrhosis, hepatic failure, and hepatocellular carcinoma [1-4]. Hepatitis B (HB) infection is an important public health problem all over the world and its infection causes more than one million deaths every year [5]. It is estimated that one third of world population have already been infected with this virus [1]. In highly endemic areas, especially in some parts of Africa and South East Asia, about 7-20% of persons are chronically infected, and more than 70% of adults show evidence of prior infection [6]. As no fully effective treatment is available, greatest emphasis is placed on prevention through immunization. Effective control of HBV transmission in areas of high and intermediate endemicity is possible only with routine vaccination of the population. Hepatitis B vaccination is one of the most effective strategies for preventing HBV infection [7]. Infant immunization against HBV has been recommended by the World Health Organization (WHO) since 1988 [8]. In 1991, WHO recommended that hepatitis B vaccination should be included in national immunization program for all countries with a hepatitis B carrier prevalence of 8% or greater by 1995 and in all other countries by 1997.

The decrease in the frequency of chronic HBV infection after the execution of infant immunization programmes has been revealed in high endemicity areas like Alaska, Taiwan, Indonesia, Polynesia, and the Gambia [9]. More than 200 million doses of plasma-derived vaccines have been distributed globally, and the safety record is impressive. The aim of the present study was to detect the immediate seroconversion following HBV vaccination of children 1 month after the last dose for a community vaccinated with Engerix^{TM}-B vaccine.

2. EXPERIMENTALS

This comparative study was conducted on infants (< one year of age) selected from four healthcare centers (two rural and two from urban areas) of the Rawalpindi. The data was collected from those infants who were brought to vaccination centers during Jan, 2011 to June 2011. The data was collected with the help of questionnaire keeping in view some variables such as age, sex, height, weight, demographic and medical history. All the newborns of age less than two months and previously not vaccinated were included in the study. Infants who were receiving immunosuppressive therapy, steroid therapy or blood and blood products were not considered for the project. Furthermore, infants with some known chronic disease were also not eligible for the study.

2.1 Ethical Considerations

Written informed consent was obtained from parents/guardians of subjects, prior to conducting any study-specific procedure.

2.2 Serological Testing

The serological testing was done in the Virology Laboratory of NIH (WHO Collaboration Centre for viral diagnostic and research). The experimental design comprised of pre-vaccination sampling, to make a base-line by collecting the samples from those subjects who have not received any dose of Hepatitis B vaccine and the post-vaccination sampling to evaluate the antibody response of the subjects after receiving the three doses of Hepatitis B vaccine. 3 mL/cc blood of infants was collected using the disposable butterfly needles of JMS 24G under the aseptic conditions with the help of an expert. Blood was immediately transferred to appropriate containers, which were already labeled with unique identification number assigned to each of the subject and this number was also mentioned at the top of the Questionnaire. Samples were then allowed to clot at room temperature for 30-45 minutes. Serum was separated by centrifugation at 3,000 rpm for 5 minutes assayed for hepatitis B surface antibodies (anti-HBs), by enzyme linked immunosorbent assay (ELISA) using quantitative panel test (Abbot Laboratories, North Chicago,

IL, USA), antibody titer was expressed in International Units per liter (IU/L) Initial serum examinations for anti-HBs was done for all recruited infants (400 infants) before starting their vaccination schedule. Only seronegative subjects for anti-HBs were further followed up. Three successive doses (10 µg) of hepatitis B recombinant vaccine Engerix^{TM}-B were given intramuscularly to all recruited infants at 2, 4 and 6 months of age. Follow-up blood samples were tested for anti-HBs after one month from the third dose of vaccine.

The minimal protective titer has been assumed almost universally to be 10 IU/L [10]. Antibody response to the vaccine (anti-HBs) was classified as good response (>100 IU/L), moderate response (10-100 IU/L) and no or poor response (<10 IU/L) [11,12]. The antibody level of 10 IU/L [19] was considered the discriminant between protective and non-protective response [13].

2.3 Statistical Analysis

All assays were conducted in triplicate and statistical analysis was done with CoStat (CoHort Software) with $p<0.05$.

3. RESULTS

Keeping in view all the specifications mentioned in the materials and methods out of 400 subjects included in the study 88 (22%) were found to be seropositive while the remaining 312 (78%) were seronegative. The baseline results are summarized in Table 1.

Table 1. Pre-vaccination seroprevalence of anti-HBs in rural and urban areas of Rawalpindi

Please insert row title	Total	Location	
		Urban	Rural
Baseline seropositive	88 (22%)	47 (53.40%)	41 (46.59%)
Baseline seronegative	312 (78%)	153 (76.5%)	159 (79.5%)

The number of blood samples included in the baseline survey remained 312 out of 400. In follow up out of 312 baseline negative blood samples, 203 were captured for the 2nd sample and 4 blood samples could not be analyzed because of the loss of sera. The attrition rate was 36%. Afterwards, 64% of the baseline subjects were analyzed for the seropositivity of the vaccine. A total of 199 samples were analyzed for the quantitative determination of anti HBs in response to three doses of hepatitis B vaccine. Immunization was well tolerated by all the infants, and no serious adverse event was reported. The seroconversion rate was found to be 93% among the infants of urban areas and 91.9% among those of rural areas. The overall seroconversion rate was 92.45%. The post vaccination seroconversion results are summarized in the Table 2. The higher post vaccination antibody titer i.e.≥1000 IU/L was shown by 30.15% of infants. The post vaccination titers results are summarized in Table 3.

4. DISCUSSION

It is usually accepted that an anti-HBs concentration above 10 IU/L is symbolic of either resolution of a past infection or positive response to vaccination. In both type, acquired immunity to type B viral hepatitis may be assumed. Antibody concentration below 10 IU/L is indicative of lack of acquired immunity, as a level of 10 IU/L is considered as the lower limit of protection [14].

Two variables were found to be significantly associated with inadequate levels of antibodies: high weight and male gender (P<0.05). The females showed more high titers of antiHBs against vaccination as compared to that of males. However, the frequency of responders and non-responders was the same for both the sexes. In this study no significant difference is found in the frequency of responders and non-responders of rural (responders=91.9% and non-responders=8.1%) and urban areas (responders=93% and non-responders=7%) of Rawalpindi Division. However, it was found that the infants of urban areas showed higher titers (no. of infants having titers ≥1000 IU/L=32) as compared to that of the rural areas (no. of infants having titers ≥1000 IU/L=28). As in urban areas the socioeconomic status is better as well as mothers are better aware of the importance of the proper nutrition and balanced diet for their child as well as for themselves.

The overall sero-protectivity rate (anti-HBs titer >10 IU/L) among the vaccinated individuals was found to be 92%, Table 2. Two separate studies conducted in South Africa and Brazil reported the antibody response among the HB vaccinated individuals as 86.8% and 90% respectively [15,16].

Table 2. The sex and location wise distribution of responders and non-responders

Post vaccination seroconversion	Total	Location		p-value	Gender		p-value
		Urban	Rural		Male	Female	
Responders	184 (92.46%)	93 (93%)	91 (91.9%)	<0.05	97 (91.5%)	87 (93.54%)	<0.05
Non-responders	15 (7.54%)	7 (7%)	8 (8.1%)	<0.05	9 (8.4%)	6 (6.45%)	<0.05

Table 3. The sex and location wise distribution of frequency of post-vaccination antiHBs antibody titer

Post vaccination anti HBs titers	Total frequency	Location		p-value	Gender		p-value
		Urban areas	Rural areas		Male	Female	
<10	15 (8.04%)	7 (8%)	8 (8.08%)	<0.05	9 (8.4%)	6 (7.52%)	<0.05
10-100	70 (35.17%)	35 (35%)	35 (35.5%)	<0.05	58 (54.71%)	12 (12.90%)	<0.05
100-500	53 (26.63%)	25 (25%)	28 (28.28%)	<0.05	23 (21.69%)	30 (32.25%)	<0.05
500->1000	61 (30.15%)	33 (32%)	28 (28.28%)	<0.05	16 (15.09%)	45(47.31%)	<0.05

Further, a study conducted in Bangladesh among EPI-vaccinated children, the rate of sero-protectivity was found to be 92.2% [17]. So, as far as the seroconversion after HB vaccine is concerned, the results of the present study are well consistent with that of others.

In the present study, among 400 vaccinated individuals (sample collected within 1 to 6 months after completion of vaccination series), 184 (92.46%) were good responders and 15 (7.53%) were non-responders respectively Table 2. Several studies have documented the percentage of non-responder between 11.9% and 21% among the vaccines in the different parts of the world [18,19]. The percentage of non-responder in this study is well consistent with other studies. All results are statistically significant as analyzed with statistical software.

5. CONCLUSION

In conclusion, this prospective study reinforces that the Hepatitis B vaccine, used in this study has a good tolerability and is highly immunogenic among infants. It is recommended that serosurvey of HBsAg and vaccine coverage at country level should be in common practice.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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