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Research Article

Observational Study About The Effectiveness of Zero-Dose HBV Vaccine on Prevention of HBV Breakthrough Infection Among Vaccinated Egyptian Infants

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ABSTRACT

Background: The implementation of universal hepatitis B virus vaccination programs had led to significant reduction in the incidence of acute and chronic HB infection, liver cirrhosis and hepatocellular carcinoma. However, this success is being threatened by the discovery of HBV vaccine breakthrough infection and vaccine associated nonresponse.

Aim: Of this cross-sectional study was to evaluate the effectiveness of zero dose HBV vaccine on prevention of HBV breakthrough infection among vaccinated Egyptian infants and to study the role of IL4 SNPs as a cause of vaccine non-responsiveness.

Methods: The present study was carried out on 77 infants their age ranged from 6 to 12 months; all of them received HBV vaccine according to the new schedule as the Egyptian Public Health Authority recommend, the first dose was administered within the first 24 hours after delivery. Their serum samples were screened for the presence of HBsAg and HBV-DNA as markers of infectivity. Their HBsAb level was detected to assess their immunity and Cytokine gene analysis to identify IL4 gene polymorphism associated with non-responsiveness to HBV vaccination.

Results: It was found that all infants were negative for HBV DNA but HBsAg was positive in 61 out of 77 (79.2%). HBsAg positivity increases after the fourth dose of vaccination and persists for two, four and even six months after vaccination in23.4%, 9.1% and 11.7% of vaccinated infants respectively. There was positive correlation between HBsAg positivity and HBs antibody titer as HBsAg positivity was 60% in non-responders, 77% in low responders and 81% in high responders, there was no statistically significant association between HBsAg positivity and the mode of delivery. The presence of IL4 SNPs especially SNP3 is correlated with the non and low responders to HBV vaccine, however this correlation is statistically non-significant.

Conclusion: HBV infection rates have significantly reduced after the introduction of the zero-dose vaccination. The presence and persistence of positive HBsAg with negative HBV-DNA after vaccination may be vaccine induced and not HBV breakthrough infection.

Keywords: Egyptian infants; HBV vaccine; zero doses; Effectiveness; HBsAg positivity; IL4 SNPs

INTRODUCTION

Hepatitis B virus (HBV) infection is a global problem that impacts affected individual's health and society. HBV is the most prevalent and the main infectious agent leading to liver disease [1]. It is estimated that around 240 million people were chronically infected with HBV worldwide and approximately 780,000 die per year as a consequence of acute disease and chronic complications, such as cirrhosis and hepatocellular carcinoma [2].

In HBV endemic areas mother to infant transmission of infection postnatal or prenatal during delivery is frequent around 90% of infants acquire HBV infection if maternal serum

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is hepatitis- Be antigen positive. Introduction of a safe, effective hepatitis B vaccine had led to universal infant vaccination, resulting in a reduced rate of perinatal HBV infection from infected mothers and to decrease HBV- related morbidity and mortality. Despite vaccination, infection persists in some of these infants and increases the incidence of HBV reservoir worldwide [3]. In Egypt HBV vaccination was conducted at 2, 4 & 6 months of age schedule and since 2016 it is conducted at 0, 2, 4 and 6-months schedule and the first dose are recommended to be given within the first day after birth (Egyptian Public Health Authority 2016 [4]. Hepatitis B vaccine for children consists of three doses started within 24 hours of birth followed by other doses of hepatitis B vaccines at intervals of at least 4 weeks [5]. In South Korea, HBV vaccination was introduced for schoolchildren in 1988 and was added to the national immunization schedule in 1991. Since 1995, routine HBV vaccination has followed routinely the schedule of a dose at 0, 1, and 6 months after birth [6,7].

A concentration of anti- HBs 1-2 months after full vaccination doses ≥ 10 IU/ml is generally accepted as offering protection. Non- responders (NR) are defined as a person who has antibody titer less than 10 mIU/ml after 1-6 months from the three doses regimen [8]. Sero-conversion in healthy individuals is as high as 90%- 100% [9]. In Egypt, about 65% of children aged from 2-4 years, were good responders to HBV vaccination having the protective level HBsAb [10]. Also, it was reported that 5-10% of the vaccinee showed an insufficient antibody response after primary vaccination with the routine schedule of triple doses of r-HBsAg vaccine, in addition to 3-20% of non-responders may not be protected from subsequent exposure to HBV infection [11-13]. Also, poor responders were 28% and non-responders were 7% among children aged 2-4 years in Egypt. The reduction in immunogenicity may be related to determinants that include host, vaccine and genetic factors [2]. Host factors that can lead to ineffective vaccination include prenatal infection, a high viremia in the maternal circulation, vaccine escape mutants, reactivation of the virus, a compromised immune system and premature birth. Other causative factors include inappropriate storage and transportation of the vaccine and improper timing, interval, or site of vaccination [8]. Numerous studies had confirmed that the body´s response after vaccination may be affected by the genetic background of the immune system [1]. Non- or hypo –response to the HBV vaccine was previously found to be associated with deletion in gene in human leukocyte antigen complexes or deficits in antigen–presenting cell (APC) function [11]. In addition single nucleotide polymorphism (SNPs)in human leukocyte antigen (HLA), tumor necrosis factor (TNF), interferon (IFN), interleukin (IL)-1, IL-4, IL-10, IL-15 and corresponding genes are associated with HBV vaccine failure [12]. An increasing number of studies had shown a significant correlation between immune response to HBV vaccine and the SNPs of immune-regulatory cytokine genes such as IL-β, IL-4, IL-10, IL-12 B, IL-13 [13]. The aim of this cross-sectional study was to evaluate the effectiveness of zero dose HBV vaccine on prevention of HBV breakthrough infection and on HBs antibody response among vaccinated Egyptian infants and to study the role of IL4SNPs as a cause of vaccine non-responsiveness.

METHODOLOGY

The present cross-sectional study was conducted from May 2017 to February 2018 at the Medical Microbiology & Immunology Department, Immunology Unit, Pediatric department, Clinical Pathology Department, Faculty of Medicine & the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Subjects and samples

Seventy-seven blood samples from vaccinated children were collected (after receiving an informed consent from their parents) they included infants aged from 6 to 12 months who had received zero dose of HBV vaccine followed by the full three compulsory doses of HBV vaccine(Hepatitis B vaccine (rDNA) Serum institute of India PVT LTD, 212/2 Hadapsar, Pune 411028, India. Each dose of 0.5 ml contains 10 mcg of purified hepatitis B surface antigen). The participated infants had received HBV vaccine at 2, 4, and 6 months of age, according to the compulsory vaccine schedule of the Egyptian Ministry of Health Population (MOHP), in addition to the zero dose of HBV vaccine which has been applied since 2016 to be administered within the first 24 hours after birth.

HBV serological testing

Blood samples of nearly 3–5 ml were aspirated under complete aseptic conditions from each participant. Serum samples were aliquoted into labeled sterile cryo tubes and stored at -20°C until being used. Qualitative determination of hepatitis B surface antigen (HBsAg) was carried out, using an enzyme-linked immunoassay kit (ELISA; Precheck, USA) the manufacturer's guidelines were followed. Quantitative detection of serum anti-HBs was conducted using an enzyme-linked immunoassays kit (ELISA; DiaSorin, Qiagen, Germany) the manufacturer's guidelines was followed. The serum anti-HBs antibody titer was analyzed after the 4th dose of vaccination. According to the international standards, anti-HBs of at least 10 IU/l were considered to give protection against HBV infection. Low responders were the vaccinated children who developed anti-HBs level between 10 and 100 IU/l after the full vaccination dose and those with anti-HBs level more than 100 IU/l as high responders.

HBV-DNA detection

Serum samples that were positive for HBsAg were screened for the detection of HBV-DNA by Polymerase chain reaction (PCR). Serum DNA was extracted using the Qiagen DNA Blood mini kit, according to the protocol of the manufacturer (Qiagen, USA). Extracted DNA was subjected to HBV-DNA detection using the universal primer pairs P1 sense and S 1-2 antisense to amplify the conserved regions of the pre-S1 and S-gene (1063 bases). The reaction mixture contained 5 µL of extracted DNA in 25 µL 1×PCR buffer containing 1.5 MgCl2, 5 pmol of each primer completed 200 µmol/L of each of the four deoxynucleotides, 1U of AmpliTaq Gold DNA polymerase and completed to 50 µl with RNAase free sterile water. The samples were incubated at 95ºC for 10 min, followed by 40 amplification cycles of 94ºC for 20 sec (denaturation), 55ºC for 20 sec

(annealing), 72ºC for 1 min (extension), and then followed by further extension at 72ºC for 10 min. After that the product was kept at 4ºC. PCR products were visualized by electrophoreses on 3% agarose gel, compared to a 50 base-pair DNA marker.

RESULTS

A total of 77 infants (41females and 36 males) were enrolled in the present study. All of them were HBV vaccinated on a 0, 2, 4- and 6-months schedule, the first dose is recommended to be given within the first day after birth. The mode of delivery was normal vaginal delivery in 33 and cesarean section in 44 infants. All serum samples were negative for HBV -DNA by PCR (Table 1). According to anti-HB titer infants were divided into three subgroups: <10 mIU/ ml (non-responders, NR), 10-100 mIU/ml (low responders, LR) and ≥ 100 mIU/ml (high responders). Among the 77 infants, 5 (6.5%), 28 (37.7%), 44 (57.1%) were in the NR, LR, and HR groups respectively.

Gene	SNP		Forword (5'-3')	Reverse (5'-3')
IL- 4	rs2243250	c.-589C>T	CTTGCCA AGGGCTT CCTTAT	CAGTCCT CCTGGG GAAAGAT
	rs2070874	c.-33C>T	CCTGTTT GTGAGGC ATTTTT	CTGGAGA GATGGTG CCAGAT
	rs2227284	c.-183+2527 T>G	TTTTTAGT ATCTCTA AGTTGGG TAGCA	GGTTCTT GACCAGC CTCACT

Table 1: Target gene –specific primer pairs for IL- 4gene.

There were also non statistical association between the age or sex of the studied infants and HBsAg positivity as the P value was 0.068 and 0.944 respectively. It was also noticed that HBsAg positivity increases gradually after the 4th dose of vaccination and this increase had two peaks one at age of 8 months 23.4% of the studied infants then decreases gradually till the 9th month as it reaches 7.8% level then starts to increase again by the age of 11th months to 18.2% of the studied infants then decrease again at 12th to 11.7% (Figure 1).

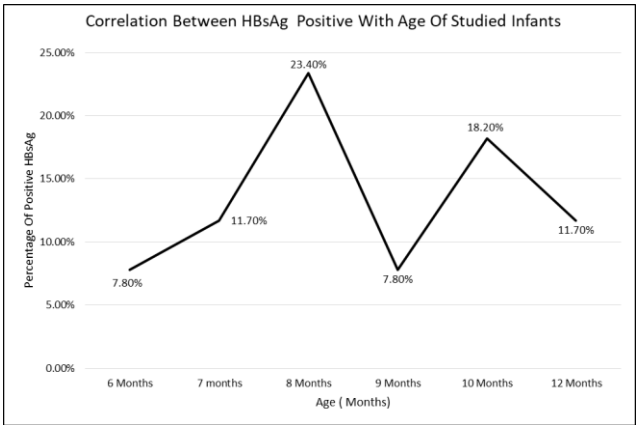

Figure 1: Correlation between HBsAg positivity with age of studied infants HBsAg positivity increases gradually after the 4th dose of vaccination and this increase had two peaks.

DISCUSSION

The implementation of universal hepatitis B virus vaccination programs had led to significant reduction in the incidence of acute and chronic HB infection, liver cirrhosis and hepatocellular carcinoma. However, this success is being threatened by the discovery of HBV vaccine breakthrough infection caused by the S gene mutants of HBV, high viral load of maternal blood and immunosuppression induced by a virus [14]. HBV vaccine breakthrough infection is an infection caused by HBV in an individual with validated history of full primary hepatitis B vaccination and who is HBsAg-, anti-hepatitis B core antigen (anti-HBc)-or HBV DNA-positive regardless of the sero-status of anti-HBs. The present cross-sectional study was carried out on 77 infants their age ranged from 6 to 12 months; all of them received HBV vaccine according to the new schedule as the Egyptian Public Health Authority recommend. According to this schedule the first dose of vaccine was administered within the first 24 hours after delivery. Their serum samples were screened for the presence of HBsAg and HBV-DNA as markers of infectivity. Also, their HBsAb level was detected to assess their immunity. It was found that all infants were negative for HBV DNA but HBsAg was positive in 61out of 77 (79.2%). The presence of positive HBsAg with negative HBV- DNA may indicate that this antigen positivity may be vaccine induced and not HBV breakthrough infection. It was found also that HBsAg positivity increases after the fourth dose of vaccination and persists for two, four and even six months after vaccination in 23.4%, 9.1% and 11.7% of vaccinated infants respectively (Figure1). These results were correlated with that of Kloster [15] who reported that nine cases of transient, confirmed antigenemia was detected in healthy individuals who donated blood 1 to 3 days following vaccination with a recombinant hepatitis B vaccine. Follow-up testing showed no evidence of infection by hepatitis B virus. They mentioned that individuals recently vaccinated for hepatitis B may still positive when tested for HBsAg. Also Mohan et al [16] has reported that they detected incidentally a hemodialysis patient with a transient hepatitis B surface antigenemia after the second dose of Hepatitis B vaccine. Another study [17] has detected that the vaccine-induced false positive HBsAgwas 50% and the duration of the positivity lasted no more than two weeks, they concluded that individuals recently vaccinated for hepatitis B may test positive for HBsAg,they follow a vaccination schedule of 0, 1, 6 months. But in the present study HBsAg persists for longer period after vaccinationand the schedule of 0, 2, 4, 6 doses was followed. We couldn't explain why this antigen persists in their serum may be the schedule used is the cause of this persistence.

Another observation was noticed in this study which is the positive correlation between HBsAg positivity and HBs antibody titer as HBsAg positivity was 60% in non-responders, 77% in low responders and 81% in high responders. This indicates that HBsAg persistence stimulates their immune response to produce antibody and low expression of HBsAg lead to low or non - response. In contrast to this the present study has found hat

from 5655 children analyzed five children (3 boys and 2 girls; 0.09%) had positive HBsAg test results. All of them had negative HBsAb titers and each of their mothers was a confirmed HBV carrier. And also, to the study done on 111 infants who received three doses of vaccine at months 0, 2, 9 months and 156 infants received three doses of vaccine at months 2, 4, 9 the scheme starting at the end of second month of life yielded a significantly higher immunogenicity than that starting at birth.

CONCLUSION

From the present study it was concluded that: The implementation of universal HBV vaccination programs has significantly reduced HBV infection rates especially after the introduction of the zero dose. The presence and persistence of positive HBsAg with negative HBV- DNA for two, four and even six months after vaccination is most probably vaccine induced and not HBV breakthrough infection. The occurrence of post-vaccination HBsAg positivity needs to be fully acknowledged to prevent misdiagnosis. There is positive correlation between HBsAg positivity and HBs antibody titer. The presence of IL4 SNPs especially SNP3 is correlated with the non and low responders to HBV vaccine.

RECOMMENDATION

This study was a preliminary study and the obtained results need to be verified by a large-scale study on a larger group of children and after longer period of time post vaccination.

CONFLICT OF INTEREST

The authors declare that they do not have any conflicts of interest.

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