Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology,
E-ISSN (2090-2506)
Volume 31 (4), October, 2024
Pages: 66–75.
www.Ejimmunology.org
https://doi.org/10.55133/eji.310407

Effectiveness of zero dose HBV vaccine on prevention of HBV breakthrough infection among vaccinated Egyptian children

Asmaa M. El-Nasser[1,5], Amany M. Tawfeik[1,5], Eman A. E. Abushady[1], Eman A. Mohammad[1], Marwa Elhady[2], Abeer M. Abdul-Mohymen[3], and Salah E. I. Ali[4]

[1]Department of Medical Microbiology & Immunology, Faculty of Medicine (for girls), Al-Azhar University, Cairo, Egypt.

[2]Department of Pediatric, Faculty of Medicine (for girls), Al-Azhar University, Cairo, Egypt.

[3]Department of Clinical Pathology, Faculty of Medicine (for girls), Al-Azhar University, Cairo, Egypt.

[4]Department of Clinical pathology, Faculty of Medicine Zagazig University, Zagazig, Egypt.

[5]Department of Medical Microbiology & Immunology, Faculty of Medicine, Badr University in Cairo (BUC), Cairo, Egypt.

[6]Pediatric Department, Faculty of Medicine, Prince Sattam ben Abdulaziz University, KSA.

Corresponding author: Eman A. E. Abu shady, Department of Medical Microbiology & Immunology, Faculty of Medicine (for girls), Al-Azhar University, Cairo, Egypt.
Email: emanabdelfatah.micro@azhar.edu.eg
eman_abushady@yahoo.ie

Abstract

Hepatitis B virus (HBV) is a vaccine preventable disease. Sufficient post vaccination response is critical step to achieve infection eradication. Vaccine hypo-responsiveness is a major risk factor for HBV chronic infection. We aimed to evaluate the effectiveness of birth dose HBV vaccine in preventing perinatal HBV infection and to detect the rate of HBV surface antibody (HBs-Ab) seroconversion and its relation to interleukin-4 polymorphism (IL-4 PM) among a group of vaccinated Egyptian infants. This observational analytical study involved 77 infants aged 6 to 12 months who received 4 doses of HBV vaccine including a zero dose. We measured serum levels of HBV-DNA and hepatitis B surface antigen (HBsAg) as markers of infectivity, and the level of (HBsAb) to assess vaccine responsiveness. Cytokine gene analysis to detect IL-4 gene polymorphism and its association with vaccine un-responsiveness were investigated. We observed that none of the vaccinated infants acquired HBV infection. Of the included 77 infants, seroconversion against HBV was detected in 72 (93.5%), 28 (36.4%) had low response and 44 (57.1%) had high response. While 5 (6.5%) were non responders. There was significant association between IL-4 gene polymorphism and the poor seroconversion after HBV vaccination. (p=0.03). Furthermore, HBsAb titer was significantly lower in children who have IL-4 gene polymorphism (p=0.014). In conclusion, implementation of birth-dose HBV vaccination is effective for prevention of perinatal infection, but seroconversion rate may be insufficient to induce long term protection. IL-4 gene polymorphism is associated with poor response to HBV vaccine.

Keywords Hepatitis B, vaccination, birth dose, seroconversion, interleukin-4 polymorphism.

Date received: 25 April 2024; **accepted:** 08 August 2024

Introduction

Hepatitis B viral infection (HBV) is a vaccine preventable disease. However, despite development of HBV vaccine, hepatitis B infection represents a worldwide health problem adversely affects both individual's health and community. Chronic HBV infection has emerged as a major risk factor for liver cirrhosis and hepatocellular carcinoma.[1] On April 10th, 2024, the World Health Organization (WHO) stated that around 254 million individuals had chronic HBV infections in 2022, with 1.2 million new cases annually globally, leading to roughly 1.1 million deaths that year.[2] Chronic HBV infection is prevalent in African countries. About 38% of pregnant African women have hepatitis B 'e' antigen (HBeAg) that expose their infants to great risk of infection transmission.[3] In Egypt, according to the Egypt Health Issues Survey (EHIS) conducted in 2015, the prevalence of chronic HBV infection among the population of Egypt aged 1 to 59 years was 1.0%.[4]

Mother to infant transmission of HBV either perinatally or during early childhood represent a major rout of infection that account for up to 50% of disease transmission leading to subsequent chronic HBV infections.[5,6] Younger age at infection is estimated as a major risk factor for infection chronicity, as up to 90% of those who had acquired infection early in infancy progress to chronic infection.[7] So early HBV vaccine administration is a key step to achieve proper protection against both perinatal and horizontal transmission of HBV infection especially in high-risk countries with low resources. Administration of the vaccine early in infancy especially when given within the first 24 hours after birth leads to decreased HBV perinatal infection and subsequently decrease HBV related morbidity and mortality.[8]

HBV vaccination is scheduled into the national immunization programs worldwide. However, there is variation in the vaccination practices and dose intervals across countries aiming to achieve better prevention through three or four dose series.[9] Only 12 out of 48 African countries implement HBV birth dose vaccination in the national immunization schedule.[10] In Egypt HBV vaccine was given at age 2, 4 and 6 months after birth. However, since 2016 an additional dose was introduced to be given within the first 24 hours after birth with high coverage rate, as up to 95% had completed vaccination schedule.[11] In Sub-Saharan African countries, HBV vaccine is given at 6^{th}, 10^{th}, and 14^{th} week post-natal.[12] While in other counties, HBV vaccination doses were scheduled at 0, 1, and 6 months after birth.[13] Nevertheless, despite the vaccine's overall high efficiency, some infants exhibited a poor response leading to an increased incidence of HBV reservoir worldwide. [6]

Assessment of seroconversion during infancy is important to ensure the efficacy of vaccination timing and proper adherence to vaccination schedule. Identification of factors associated with poor response to vaccination is mandatory to allow early intervention measures to be taken. [14] HBs antibody titer ≥10 IU/ml indicate sufficient vaccination response while low titer <10 IU/ml reflect un-responsiveness. Furthermore, vaccination response is categorized into high response in those with antibody titer > 100 mIU/ml indicating long term protection against HBV infection while those antibody titer between 10 and 100 mIU/ml are considered as hypo-responsive and less protected making them at greater risk for infection later on and need booster dose immunization.[15]

Post vaccination seroconversion and immune response depend on complex interaction between host, vaccine and genetic factors.[16] Host factors include gestational age, immunity, vaccine escape mutants and maternal infection. Vaccine related factors include unsuitable storage and transportation environment and inappropriate timing or intervals of vaccination doses.[17] Genetic background of the immune system plays a key role in individual response to vaccination.[18] Previous reports linked poor response to HBV vaccine to genetic polymorphism, deletion at human leukocyte antigen complexes, or deficits in antigen presenting cell function.[19,20] Emerging evidences demonstrated a significant correlation between immune response to HBV

vaccine and the single nucleotide polymorphism (SNPs) of immunoregulatory cytokine genes.[21]

Interleukin-4 (IL-4) is a multifunctional cytokine released from T helper 2 lymphocytes and has regulatory role on immune response to vaccination. IL-4 gene polymorphism is associated with alteration in HBV vaccine response; however, this effect varies among different population.[22] No sufficient data are available regarding the impact of HBV immune response and IL-4 gene polymorphism among African and Middle East population. The current study aimed to evaluate the effectiveness of birth dose HBV vaccine in preventing perinatal HBV infection and detect the rate of HBs antibody seroconversion and its relation to IL-4 polymorphism among a group of vaccinated Egyptian infants who completed 4-dose immunization schedule including birth dose immunization.

Patients and Methods

This cross-sectional observational analytical study involved 77 infants who were selected consecutively from outpatient pediatric department of Al-Zahraa university hospital, Cairo, Egypt. The study was conducted during the period from January 2020 to May 2020. Inclusion criteria included healthy infants aged 6 to 12 month of both sexes who completed four HBV vaccine schedule including a birth dose within the first 24 hours after delivery. Exclusion criteria included infants who have congenital malformation, protein energy malnutrition, infant who has systemic diseases (cardiac, renal, respiratory, hematological or hepatic based on history and examination, infant who were born preterm (<37 weeks gestation), intrauterine growth retardation and infant received any medications impair their immune system as corticosteroid or chemotherapy.

Laboratory investigations were performed at the Immunology unit of the Department of Medical Microbiology and Immunology, and the Department of Clinical Pathology, Faculty of Medicine, and the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Medical history and Examination

Parents of participating infants in the study were asked to provide infant detailed history including age, sex, sociodemographic data, gestational age, perinatal history, dietetic history, past medical history, manifestations suggestive of systemic diseases and family history of hepatitis virus infections either B or C, vaccination history with special emphasis on HBV vaccination schedule, birth dose, timing of subsequent doses and dose intervals. All included infants received four doses of HBV vaccine according to the compulsory vaccine schedule of the Egyptian Ministry of Health and Population. This included a birth dose within the first 24 hours after delivery. All included infants were of the same socioeconomic standard receiving ordinary traditional food and vitamin D supplementation prophylactic dose. Maternal screening for HBV status was not performed. Thoroughly general and systemic clinical examination was performed with special emphasis on anthropometric measurement and nutritional status.

HBV serological testing

A venous blood sample (3-5 ml) was withdrawn under complete aseptic conditions from each study infant. Blood samples were centrifuged, and serum was collected into two labeled sterile Cryogenic tubes (Thermo Fischer Scientific, USA), and stored at -20°C until used.

Qualitative detection of HBsAg was performed, using enzyme linked immunoassay (ELISA) kits (CA 92801, Precheck, USA), according to the manufacturer instructions. Quantitative measurement of serum HB surface antibody (HBsAb) was performed using ELISA kits (DiaSorin, Qiagen, Germany), according to the manufacturer instructions. Seroconversion was considered in infants having HBsAb titer ≥10 IU/l. However, infants were categorized into low responders if HBsAb titer was between 10 and 100 IU/l and high responders if HBsAb titer was >100 IU/l. While those with HBsAb titer <10 IU/l were considered non-responders.[14]

HBV-DNA detection

HBV-DNA was detected using the polymerase chain reaction (PCR). DNA was extracted using Qiagen DNA Blood mini kits (Qiagen, USA). HBV-DNA detection from extracted DNA was performed 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 sequence of the universal primer pairs was: P1 sense (5'TCACCATATTCTTGGGAACAAGA 3') (2823-2845 nt) and S 1-2 antisense (5'CGAACCACTGAACAAATGGC 3') (704- 685 nt).

The reaction mixture was contained in 50 µl tubes. It contained 5 µL of extracted DNA in 25 µL 1 × PCR buffer containing 1.5MgCl2, 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 95°C for 10 min, followed by 40 amplification cycles, each of 94°C for 20 sec (denaturation), 55°C for 20 sec (annealing), 72°C for 1 min (extension), and then followed by further extension at 72°C for 10 min. After that the product was kept at 4°C. PCR products were visualized by electrophoreses on 3% agarose gel, compared to a 50 base-pair DNA marker.

Cytokine gene analysis

Due to high financial cost, cytokine gene analysis was performed for selected cases. Based on the detected seroconversion; 15 serum samples of infants with different response rate of matched age, sex and vaccination timing were selected for cytokine gene analysis to detect the IL-4 gene polymorphism. They were 5 non-responders who had HBsAb titer <10 IU/ml, 5 low responders who had HBsAb titer between 10-100 IU/ml and 5 high responders with HBsAb titer > 100 IU/ ml.

Three single-nucleotide polymorphism *(*SNP*)* sites for the IL-4 gene (rs2243250, rs2070874, and rs2227284) were detected by GoTaq Q PCR master mix SYBR green Promega kit (Lot NO. 0000213293, Promega, USA) and analyzed using the real time PCR instrument (DT Lite-4, from DNA-Technology, Russia) that is used for qualitative and quantitative analysis of DNA and RNA targets. Genomic DNA was extracted from serum samples using the Qiagen DNA Blood mini kit (Qiagen, USA), according to the protocol of the manufacturer. PCR amplification was carried out using sequence-specific primers (Table 1) according to the following thermal profile: 94°C for 5 min, followed by 35 cycles each of 94°C for 30 s, 58°C for 30 s, and 72°C for 50 s for, followed by72°C for 10 min.

Statistical Analysis

Data were collected and statistically analyzed using the statistical package for social sciences (SPSS) version 21 (IBM, USA). Data were expressed as number and percentage for qualitative data and mean, standard deviation (SD), median and Interquartile Range (IQR) for numerical data. Comparisons between groups were done by Chi-square test for qualitative data, independent t test for numerical data of normal distribution and Mann Whitney test for numerical data if normal distribution is not assumed. The Pearson Correlation was used to test for the strength of association between two continuous variables. The level of significance was set at *p*-value <0.05.

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

Gene	SNP		Primers sequence (5'-3')
IL- 4	rs2243250	c.-589C>T	F: CTTGCCAAGGGCTTCCTTAT R: CAGTCCTCCTGGGGAAAGAT
	rs2070874	c.-33C>T	F: CCTGTTTGTGAGGCATTTTT R: CTGGAGAGATGGTGCCAGAT
	rs2227284	c.-183+ 2527T>G	F: TTTTTAGTATCTCTAAGTTGGGTAGCA R: GGTTCTTGACCAGCCTCACT

Results

A total of 77 infants were enrolled in the present study. Their age ranged between 6-12 months with a mean age of 8.94 ± 1.86 months. They were 41 females and 36 males. None of them had hepatitis B infection as all serum samples were negative for HBV-DNA by PCR. HBsAg was detected in sera of 79.2% of study infants. Seroconversion against hepatitis B was detected in 72 (93.5%) of included infants. Of these, 28 (37.7%) had low response and 44 (57.1%) had high response, while the remaining 5 (6.5%) were seronegative, as shown in Table 2.

Table 2. Demographic and laboratory data of the 77 studied Infants.

Studied parameters		Results
Age (months)	Mean ± SD	8.94 ± 1.86
	Range	6 – 12
Sex	Female	41 (53.2%)
	Male	36 (46.8%)
Age distribution at time of assessment	6-8 month	38 (49.3%)
	9-10 month	27 (35.1%)
	11-12 month	12 (15.6)
HBsAb level (IU/L)	Mean ± SD	371.4 ± 139.1
	Range	0.5 - 601.5
	Median (IQR)	315.1 (184.4)
HB seroconversion	No-seroconversion	5 (6.5%)
	Sero-conversion	72 (93.5%)
HBsAb response	Non responders	5 (6.5%)
	Low responders	28 (37.7%)
	High responders	44 (57.1%)
HBsAg	Negative	16 (20.8%)
	Positive	61 (79.2%)
HBV-DNA	Negative	77 (100.0%)

There was no significant association between age at the time of assessment, sex and the frequency of seroconversion among the studied infants, as shown in Table 3. While there was significant association between IL-4 gene polymorphism and poor seroconversion after HBV vaccination, as shown in Table 4. Furthermore, HBs antibody titer was significantly lower in children who had IL-4 gene polymorphism, as shown in Table 5.

Table 3. The frequency of hepatitis B seroconversion in relation to age and sex among the 77 studied infants.

Variables		Non responders N=5	Low responders N=28	High responders N=44	*p*-value
Sex	Male	3 60%)	13 (46.4%)	17 (38.6%)	NS
	Female	2 40%)	1 5 (53.6%)	27 (61.4%)	
Age/months at assessment	Mean±SD	8.8±1.3	8.9±1.95	8.9±1.7	NS
Age distribution	6-8 m	3 (60%)	14 (50%)	21 (47.7%)	NS
	9-10 m	1 (20%)	13 (46.4%)	13 (29.5%)	
	11-12m	1 (20%)	1 (3.6%)	10 (22.7%)	
HBsAb level (IU/L)	Mean±SD	7.3±1.6	52.9±31.9	251.0±122.2	<0.0001
	Range	5.9 - 9.9	12.8 - 98.3	102.4 - 601.5	
	Median	6. 8 (2.4)	53.9 (63.9)	216.7 (260.3)	

p > 0.05 is not significant (NS).

Table 4. The relation between hepatitis B seroconversion and interleukin-4 gene polymorphism SNP in the 15 studied infants.

Interleukin - 4 (IL-4)		Non Responders N=5	Low Responders N=5	High Responders N=5	*p* value
Age /month	Mean± SD	8.8±1.3	9.3±1.9	8.8 ± 1.3	NS
Sex	Male	3 (66.7%)	2 (33.3%)	3 (66.7%)	NS
	Female	2 (33.3%)	3 (66.7%)	2 (33.3%)	
IL-4 PM	positive	3 (60%)	4 (80%)	0 (0%)	0.03
	negative	2 (40%)	1 (20%)	5 (100%)	
IL-4 SSP	SNP 1	0 (0%)	0 (0%)	0 (0%)	
	SNP 2	0 (0%)	0 (0%)	0 (0%)	
	SNP 3	2 (40%)	2 (40%)	0 (0%)	
	SNP 1,2	0 (0%)	1 (20%)	0 (0%)	
	SNP 2,3	0 (0%)	0 (0%)	0 (0%)	
	SNP 1,2,3	1 (20%)	1 (20%)	0 (0%)	

PM; polymorphism, SSP; sequencespecific primers, SNP: single nucleotide polymorphism
p > 0.05 is not significant (NS).

Table 5. Hepatitis B surface antibody level in relation interleukin-4 gene polymorphism the 15 studied infants.

		Gene polymorphism positive (N= 7)	Gene polymorphism negative (N= 8)	*p* value
Age	Months	9.2 ± 1.6	8.8 ±1.3	NS
Sex	Male/Female	4 (57.1%)/ 3 (42.9%)	4 (50%)/4 (50%)	NS
HBsAb (IU/L)	Mean ±SD	18.5±16.2	202.4±160.2	0.014
	Median (IQR)	12.8 (21.3)	221.1 (316.3)	

* HBs antibody titer was significantly lower in children who had interleukin-4 gene polymorphism.
p > 0.05 is not significant (NS).

Discussion

Eradication of HBV chronic infection gains a great concern worldwide. Vaccine hypo-responsiveness is a major risk factor for HBV chronic infection. We aimed to evaluate the effectiveness of birth dose HBV vaccine in preventing perinatal HBV infection and to detect the rate of HBV surface antibody (HBs-Ab) seroconversion and its relation to interleukin-4 polymorphism (IL-4 PM) among a group of vaccinated Egyptian infants. Introduction of a birth dose of vaccination is reported to be an effective measure for elimination of perinatal infection. However, this vaccination protection is not lifelong. Several studies recommend poster doses later in those with low HBs antibody level.[23-26]

Seroconversion rates decreased with age and there was a positive correlation between the initial HBs antibody production and the duration of protection against HBV infection. Higher rate of antibody production indicated proper immune response.[27]

From the present study it was found that none of the studied infants who received four-dose regimen of HBV vaccination developed HBV perinatal infection. These findings agreed with those reported by Li et al., 2023,[28] who indicated that the incidence of seroconversion in the booster group was significantly lower than that in the non-booster group [25.64% (10/39) vs 67.74% (63/93), $p<0.001$]. The findings of the present study were in accordance with previous reports by Dassah et al., 2015[29] and Yazdanpanah et al., 2010,[30] who found no gender differences in post HBV vaccination seroconversion.

In the present study, we found that among the studied infants the seroconversion rate

(HBsAb level >10 IU) was 93.5% after four doses of vaccinations. This result agreed with that indicated by Norouzirad et al., 2014, [31] who reported 90% seroconversion in one-year old vaccinated infants. In a previous Egyptian study of children aged less than 5-year-old, it was found that 13.6% were non responders, 39% had low response and 47.3% had high response after completing a three-dose vaccination schedule against HBV [32]. While in the present study the included infants had four-dose vaccination schedule and 6.5% of them were non responders, 37.7% had low response and 57.1% with high response. The higher rate of non-responders in the previous study could be attributed to using three doses of the vaccine, as the birth dose was not obligatory implemented, at that time, in the Egyptian vaccination schedule.

Despite achieving seroconversion, the rate of antibody production was variable suggesting that factors affecting the rate HBs antibody production is not only limited to the number of vaccine doses. The duration between vaccination dose is one of the factors that contribute to the rate of antibodies production. [33] Irregular intervals between subsequent vaccination doses after the birth dose could explain the variability in HBs antibody production among studied infants.

In the current study, there was a high percentage of those with low antibody response 37.7% with the evidence that antibody level decreased with age. Therefore, our finding raised the attention that despite giving four doses of HBV vaccination regimen, but those with low antibody response required follow up as they are in need for a booster dose of HBV vaccination later. Immune assessment of vaccination response and identification of antibody titer rather than detection of seroconversion rate is an important step to provide proper eradication and prevent HBV breakthrough infection later in life. The World Health Organization raised the concern regarding post vaccination immune persistence as there is several reports denoting the need for post vaccination booster dose even among population who received HBV vaccine at birth.[23-26, 34]

Despite the wide practice of measuring HBsAg titer to determine HBV infection, PCR for HBV-DND is the accurate method to identify infection. [35] In the current study HBs Ag was assessed among our included infants, however we cannot rely on it to determine infection as its level can persist as false positive circulating HBsAg reactivity could remain for variable durations after vaccination. The study by Bernstein et al., 1994,[36] reported post vaccination positive HBsAg in 65% infants that disappeared 18 days after vaccination. The study by Koskal et al., 1996,[37] found that 69.2% of vaccinated infants had transient post vaccination positive HBsAg for up to 28 days after vaccination. Post vaccination antigenemia developed regardless of the type of vaccine.[38] The exact mechanism for this transient HBV surface antigenemia is unclear. It may be explained by variable muscular blood flow that affects the rate of vaccine absorption from tissue leading to variable duration of persistent circulating antigen.[39,40]

Elevated HBsAg reflects vaccine induced passive expression of HBV antigen rather than infection induced virus replication. [41] Irregular intervals, delayed vaccination doses and improper timing of subsequent doses after receiving birth dose may affect the duration of clearance of HBsAg, however no sufficient data are available regarding this issue. In our study, none of the included infants had hepatitis B breakthrough infection as evaluated by HBV-DNA detection. The presence and persistence of positive HBsAg with negative HBV-DNA among the studied infants might be a vaccine related issue rather than HBV breakthrough infection. Interpretation of HBsAg reactivity in recently vaccinated infants should be done with caution with confirmation by HBV-DNA detection to establish a diagnosis of HBV infection.

The current study demonstrated a significant association between HBs antibody production and IL-4 gene polymorphism especially SNP3 (rs2243250, rs2070874, and rs2227284). In supporting this finding, the study by Roh et al., 2017,[42] concluded that IL-4 gene polymorphisms (rs2243250C and rs2227284G) may be included in the immune response to HBV vaccine. On the other hand, a meta-analysis study revealed that

IL-4 gene polymorphisms (rs2243250, rs2070874 and rs2227284) were associated with high immune response to HBV vaccine among Asian but not Caucasian populations.[43]

IL-4 is involved in both humoral and cell-mediated immune response as it increases the major histocompatibility complex class II on the B-lymphocytes leading to increased antibodies production. Mutation of IL-4 gene impairs viral antigen expression to lymphocytes leading to insufficient immune response to vaccination.[44] Identification of subjects who had IL-4 gene polymorphisms may allow prediction of vaccine immune response.[45]

The limitation of the current study included lacking data regarding maternal hepatitis B status as there was no routine assessment of pregnant mother HBV infection. Therefore, the impact of maternal viremia on vaccination response could not be evaluated among the included infants. However, the current study revealed that regardless of maternal hepatitis B infection status, birth dose of HBV vaccine within the first 24 hours after birth was an effective in preventing perinatal HBV infection. Another limitation is the small number of included infants and inability to perform genetic mutation assessment for all the included infants which was related to the high financial cost as this study was unfunded. Therefore, the current study findings may serve as a base for large scale longitudinal studies evaluating risk factors for poor response of HBV vaccination.

We conclude that implementation of a birth-dose HBV vaccination is effective for prevention of perinatal infection, but the seroconversion rate may be insufficient to induce long term protection. Interpretation of HBsAg reactivity in recently vaccinated infants should be done with caution with confirmation by HBV-DNA detection to establish a diagnosis of HBV infection. Interleukin-4 gene polymorphism is associated with poor response to HBV vaccine.

Author Contributions

EAEA, AME, EAM, ME; responsible for manuscript writing and development. AMT, SEIA; responsible for data & statistical analysis. AME, SEIA, ME, AMA; responsible for the sample recruitment and clinical records. AME, AMT, AMA; responsible for laboratory tests.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for girls, Al-Azhar University (IRB no: 201٩/23/2).

Informed consent

Caregivers of included infants were informed about the aim of the study and a written consent was obtained from each caregiver before the child was included in the study.

References

1. Nguyen, M., Wong, G., Gane, E., et al. (2020). Hepatitis B Virus: Advances in Prevention, Diagnosis, and Therapy. *Clin Microbiol Rev.* 33(2): e00046-19.
2. Easterbrook, P. J., Luhmann, N., Bajis, S., Min, M. S., Newman, M., Lesi, O., & Doherty, M. C. (2024). WHO 2024 hepatitis B guidelines: an opportunity to transform care. The Lancet Gastroenterology & Hepatology, 9(6), 493-495.
3. Schweitzer, A., Akmatov, M. and Krause, G. (2017). Hepatitis B vaccination timing: results from demographic health surveys in 47 countries. Bull World Health Organ. 95(3):199-209G
4. Abdelhamed, W., & El-Kassas, M. (2024). Hepatitis B virus in Egypt: the whole story. Egyptian Liver Journal, 14(1), 56..
5. Joshi, S.S.; Coffin, C.S. Hepatitis B and Pregnancy: Virologic and Immunologic Characteristics. Hepatol. Commun. 2020, 4, 157–171.
6. Rybicka, M. and Bielawski, K. (2020). Recent Advances in Understanding, Diagnosing, and Treating Hepatitis B Virus Infection. Microorganisms. 15; 8(9):1416.

7. Hu, Y. and Yu, H. (2020). Prevention strategies of mother-to-child transmission of hepatitis B virus (HBV) infection. *Pediatric investigations.* 4 (2): 133-137
8. Nayagam, S., Shimakawa, Y. and Lemoine, M. (2020). Mother-to-child transmission of hepatitis B: What more needs to be done to eliminate it around the world? *J. Viral Hepat.* 27:342– 349.
9. Wu, J., Li, D. and Zhou, Y. (2016). Association between timely initiation of hepatitis B vaccine and completion of the hepatitis B vaccine and national immunization program vaccine series. *Int J Infect Dis.* 51:62–5
10. Bassoum, O., Kimura, M., Tal Dia, A., et al. (2020). Coverage and Timeliness of Birth Dose Vaccination in Sub-Saharan Africa: A Systematic Review and Meta-Analysis. *Vaccines (Basel).* 8(2):301.
11. Salama, I., Sami, S., Elserougy, S., et al. (2020). Humoral Immune Memory to Hepatitis B Vaccine after Primary Vaccination of Children and Adolescents in Assiut, Egypt. *Oman Med J.* 30;35(5): e175.
12. Howell, J., Lemoine, M. and Thursz, M. (2014). Prevention of materno-foetal transmission of hepatitis B in sub-Saharan Africa: the evidence, current practice and future challenges. *J Viral Hepat.* 21(6):381–96.
13. Nelson, N., Easterbrook, P. and McMahon, B. (2016). Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. *Clin Liver Dis.* 20(4):607-628.
14. Insulander, M., Lindh, G., Stenkvist, J., et al. (2020). Long-term follow-up of a vaccination program for infants born to HBsAg-positive mothers in Stockholm County, Sweden. *Vaccine.* 22; 38(4):790-793.
15. Sahana, H., Sarala, N. and Prasad, S. (2017). Decrease in Anti-HBs Antibodies over Time in Medical Students and Healthcare Workers after Hepatitis B Vaccination. *Biomed Res Int.* 2017:1327492.
16. Furman, D. and Davis, M. (2015). New approaches to understanding the immune response to vaccination and infection. *Vaccine.* 33(40):5271-5281.
17. Zimmermann, P. and Curtis, N. (2019). Factors That Influence the Immune Response to Vaccination. *Clin Microbiol Rev.* 32(2): e00084-18.
18. Castiblanco, J. and Anaya, J. (2015). Genetics and vaccines in the era of personalized medicine. *Curr Genomics.* 16(1):47-59.
19. Okada, Y., Uno, N., Sato, S., et al. (2017). Strong influence of human leukocyte antigen-DP variants on response to hepatitis B vaccine in a Japanese population. *Vaccine.* 35:5662–5665.
20. Akcay, I., Katrinli, S., Ozdil, K., et al. (2018). Host genetic factors affecting hepatitis B infection outcomes: Insights from genome-wide association studies. *World J Gastroenterol.* 24(30):3347-3360.
21. Zhang, Z., Wang, C., Liu, Z., et al. (2019). Host Genetic Determinants of Hepatitis B Virus Infection. *Front Genet.* 10:696.
22. Lin, Y., Lan, Y., Huang, Y., et al. (2012). Effects of cytokine and cytokine receptor gene variation on high anti-HB titers: following up on Taiwan's neonatal hepatitis B immunization program. *Clin. Chim.* 413:1194–1198.
23. Romanò, L., Carsetti, R., Tozzi, A., et al. (2014). Chronic hepatitis B infection in adolescents vaccinated at birth: an alarm bell in favor of the need for a booster? *Hepatology.* 59(1):349.
24. Anderson CL, Remschmidt C, Drobnitzky FP, Falkenhorst G, Zimmermann R, Wichmann O, Harder T. Hepatitis B immune status in adolescents vaccinated during infancy: A retrospective cohort study from a pediatric practice in Germany. Hum Vaccin Immunother. 2016; 12:779–784.
25. Wang ZZ, Gao YH, Lu W, Jin CD, Zeng Y, Yan L, Ding F, Li T, Liu XE, Zhuang H. Long-term persistence in protection and response to a hepatitis B vaccine booster among adolescents immunized in infancy in the western region of China. Hum Vaccin Immunother. 2017; 13:909–915.
26. Di Lello, F. A., Martínez, A. P., & Flichman, D. M. (2022). Insights into induction of the immune response by the hepatitis B vaccine. World Journal of Gastroenterology, 28(31), 4249.
27. Said, Z. and Abdelwahab, K. (2015). Induced immunity against hepatitis B virus. *World J Hepatol.* 7(12):1660-1670.
28. Li Y, Li L, Song Y, Liu M, Zhai X, Duan Z, et al. Booster Vaccination in Infancy Reduces the Incidence of Occult HBV Infection in Maternal HBsAg-positive Children. J Clin Transl Hepatol 2023;11(3)661–669. doi: 10.14218/JCTH.2022.00213.
29. Dassah, S., Sakyi, S., Frempong, M., et al. (2015). Seroconversion of Hepatitis B Vaccine in Young Children in the Kassena Nankana District of Ghana: A Cross-Sectional Study. *PLoS One.* 10(12): e0145209.

30. Yazdanpanah, B., Safari, M. and Yazdanpanah, S. (2010). Persistence of HBV vaccine's protection and response to hepatitis B booster immunization in 5-to 7-year-old children in the Kohgiloyeh and Boyerahmad Province, Iran. *Hepatitis monthly* 10: 17
31. Norouzirad, R., Shakurnia, A., Assarehzadegan, M., et al. (2014). Serum levels of anti-hepatitis B surface antibody among vaccinated population aged 1 to 18 years in Ahvaz city southwest of Iran. *Hepat Mon.* 14(1): e13625.
32. Salama, I., Sami, S., Salama, S., et al. (2014). Persistence of protection to hepatitis B vaccine and response to booster dose among children and adolescents in Dakahleya- Egypt. *Egypt J Immunol.* 21(1):13-26.
33. Osiowy, C. (2018). From infancy and beyond… ensuring a lifetime of hepatitis B virus (HBV) vaccine-induced immunity. *Hum Vaccin Immunother.* 14(8):2093-2097.
34. World Health Organization (2019). Hepatitis B vaccines: WHO position paper, July 2017 - Recommendations. *Vaccine.* 37(2):223-225.
35. Xiao, Y., Thompson, A. and Howell, J. (2020). Point-of-Care Tests for Hepatitis B: An Overview. *Cells.* 9(10):2233. doi:10.3390/cells9102233
36. Bernstein, S., Krieger, P., Puppala, B., et al. (1994). Incidence and duration of hepatitis B surface antigenemia after neonatal hepatitis B immunization. *Journal of Pediatrics.* 125(4):621–622.
37. Köksal, N., Altinkaya, N. and Perk, Y. (1996). Transient hepatitis B surface antigenemia after neonatal hepatitis B immunization. *Acta Paediatr.* 85(12):1501-2.
38. Otağ, F. (2003). False positive HBsAg result in blood donors due to administration of three different recombinant DNA Hepatitis B vaccines. *Vaccine.* 21(25–26):3734–3737.
39. Janzen, L., Minuk, G., Fast, M., et al. (1996). Vaccine-induced hepatitis B surface antigen positivity in adult hemodialysis patients: incidental and surveillance data. *Journal of the American Society of Nephrology.* 7(8):1228–1234.
40. Anjum, Q. (2014). False positive Hepatitis B Surface Antigen due to recent vaccination. *Int J Health Sci (Qassim).* 8(2):189-193.
41. Singh, S. (2007). Hepatitis B vaccine induced HBsAg positivity. *Hep B Annual.* 4:55–60.
42. Roh, E., Song, E., Yoon, J., et al. (2017). Effects of interleukin-4 and interleukin-12B gene polymorphisms on hepatitis B virus vaccination. *Ann Hepatol.* 16(1):63-70.
43. Cui, W., Sun, C., Deng, B., et al. (2013). Association of polymorphisms in the interleukin-4 gene with response to hepatitis B vaccine and susceptibility to hepatitis B virus infection: a meta-analysis. *Gene.* 525(1):35-40.
44. Jiang, H., Harris, M. and Rothman, P. (2000). IL-4/IL-13 signaling beyond JAK/STAT. *J Allergy Clin Immunol*. 105:1063-1070
45. Chen, J., Liang, Z., Lu. F., et al. (2011). Toll-like receptors and cytokines/cytokine receptors polymorphisms associate with non-response to hepatitis B vaccine. *Vaccine.* 29:706-711.