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Long-term Impact of Primary Hepatitis B Prevention on Egyptian Blood Donors: A 20-Year Follow-Up After Perinatal Vaccination

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ABSTRACT **: In this study, we aimed to evaluate the long-term persistence of antibodies against hepatitis B surface antigen (anti-HBs) and the incidence of HBV breakthrough infections in a cohort of Egyptian blood donors, 20 years following their primary vaccination. The study involved 1,500 blood donors, all of whom were born after 1992 and had undergone routine hepatitis B vaccination. Anti-HBs levels were determined using a quantitative immunoassay, and the presence of hepatitis B surface antigen (HBsAg) as well as antibodies to hepatitis B core antigen (anti-HBc) were assessed. For donors who tested positive for anti-HBc and/or HBsAg (n=8), further analysis of HBV DNA was conducted through polymerase chain reaction (PCR). The results indicated that the mean age of the participants was 20.02 ± 1.93 years, with 61% (916) being male. A significant portion of the cohort, 66.6% (999 donors), had anti-HBs levels below the protective threshold of 10 mIU/ml, with an average anti-HBs level of 33.1 ± 112.4 mIU/ml across the entire group. Additionally, eight donors (0.5%) were identified as having prior HBV exposure, as evidenced by positive anti-HBc results. Of these, seven had anti-HBs levels lower than 10 mIU/ml. Notably, one donor (12.5%) among those positive for anti-HBc exhibited signs of active HBV infection, as demonstrated by the presence of both HBsAg and detectable HBV DNA. In conclusion, 66.6% of the vaccinated blood donors exhibited non-protective anti-HBs levels two decades post-vaccination, suggesting a marked decline in immunity over time.**

Keywords: Hepatitis B vaccination, Anti-HBs persistence, HBV breakthrough infection, Long-term immunity

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I. INTRODUCTION

In 2019, an estimated 296 million people worldwide were living with chronic hepatitis B infection, with the hepatitis B virus (HBV) being the cause of approximately 820,000 deaths annually, primarily due to complications like cirrhosis and hepatocellular carcinoma (HCC) [**1**]. By 2022, thanks to widespread hepatitis B (HB) vaccination programs, the prevalence of HBV infection in children under 5 years of age had decreased to 0.7% [**2**]. In Egypt, where the prevalence of HBV was previously reported between 1.3% and 1.5%, the introduction of a national vaccination program for newborns has led to a marked reduction in infection rates [**3**]. Since 1992, Egypt's routine immunization schedule has included the hepatitis B vaccine, administered in three doses at 2, 4, and 6 months of age [**4**]. As of 2019, a birth dose of the HB vaccine is also administered within 24 hours of birth, contributing to a high vaccine coverage rate of around 97% [**5**].

Despite this early vaccination, the immunity provided by perinatal HB vaccination tends to diminish during adolescence, generally within 10–15 years after initial vaccination [**6,7**]. Studies show that approximately 62.4% of adolescents do not maintain protective anti-HBs antibody levels [**6**]. During this period, adolescents are at an increased risk of HBV exposure through activities such as sexual contact, intravenous drug use, or medical procedures. A large-scale study of 18,779 individuals indicated that despite being vaccinated, some individuals were still exposed to HBV, with anti-HBc positivity rates ranging from 0.1% to 4% [**8**]. Thus, it is

essential to determine whether HBV infection can resurface in healthy adolescents and adults years after their initial HB vaccination. The hepatitis B vaccine remains one of the most effective methods for preventing HBV infection [**9**]. In individuals with intact immune systems, protection may not solely depend on the presence of detectable antibodies [**10**]. However, in immunocompromised patients, the loss of antibodies may indicate a reduction in protection against HBV [**11–13**]. Additionally, individuals who have been vaccinated may still lose anti-HBs protection or even experience HBV reactivation under specific clinical conditions, such as organ transplantation or treatment for autoimmune, rheumatological, or malignant diseases [**14–21**]. As a preventive measure, it has been suggested to assess the risk of HBV reactivation by monitoring baseline anti-HBs levels and their progression during treatment in at-risk patients [**22–25**]. The aim of this study is to examine the long-term persistence of anti-HBs antibodies and the incidence of HBV breakthrough infections among Egyptian blood donors, two decades after their primary hepatitis B vaccination.

II. PATIENTS AND METHODS

Study Setting

This study was carried out at Zagazig University, Zagazig, Egypt, from January 2022 to December 2023. All procedures adhered to the ethical standards set by the Zagazig University Human Research Ethics Committee. Informed written consent was obtained from all participants prior to their inclusion in the study.

Study Population

The study included a total of 1,500 volunteer blood donors who had received hepatitis B (HB) vaccinations. These donors were sourced from the Egyptian Red Crescent in Tanta, Egypt (n=605), and the Regional Main Blood Bank in Seger, Tanta, Egypt (n=895). All donors were born after 1992 (between 1998 and 2007) and had completed the routine hepatitis B vaccination program. Exclusion criteria included donors who had not completed the national infant vaccination program, those infected with hepatitis C virus (HCV) or human immunodeficiency virus (HIV), or those on immunosuppressive therapy.

Vaccination Program

Egypt's universal hepatitis B vaccination program was initiated in 1992, achieving a coverage rate of 98% [3]. The program consists of three doses of recombinant HB vaccine administered at 2, 4, and 6 months of age, following the vaccination schedule established by the Egyptian Ministry of Health and Population during the study period (1998-2007).

Data Collection

The following information was collected from all donors: date of birth, age, sex, address, marital status, history of intravenous drug use, previous hospitalizations, engagement in illegal sexual activities, repeated syringe use, history of blood transfusion, tattooing, hepatitis B vaccination status, medical history (including diabetes mellitus), and family history of HBV infection. Body Mass Index (BMI) was calculated for all donors. Laboratory tests were conducted to measure serum levels of antibodies to hepatitis B surface antigen (anti-HBs), hepatitis B surface antigen (HBsAg), and antibodies to hepatitis B core antigen (anti-HBc) in all participants (n=1500). Additionally, HBV deoxyribonucleic acid (HBV-DNA) testing was performed for donors who tested positive for anti-HBc and/or HBsAg (n=8).

Blood Sample Collection

Five milliliters of venous peripheral blood were collected from each donor. After centrifugation, the serum was separated into two aliquots and stored at -40°C until further analysis.

Serological Testing

Hepatitis B surface antigen was detected using the ELISA test (Monolisa™ HBs Ag ULTRA, BIO-RAD, FS, USA). Total antibodies against HBcAg were determined using Elecsys® Anti-HBc II, while antibodies to HBsAg were measured quantitatively using Elecsys® Anti-HBs II (Roche Diagnostics, Roche Diagnostics). An anti-HBs titer of less than 10 IU/L was considered non-protective.

HBV-DNA Detection

Serum DNA was extracted using the Qiagen DNA blood mini kit, following the manufacturer's protocol (Qiagen, Valencia, CA, USA) [**26**]. Extracted DNA was subjected to nested multiplex PCR for the detection of HBV-DNA. The primer pair P1sense (5TCACCATATTCTTGGGAAACAAGA3) and S1-2 antisense (5CGAACCACTGAACAAATGGC3) was used to amplify the conserved regions of the pre-S1 and S-gene (1063 bases).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 24. Qualitative data were presented as frequencies and percentages, while quantitative data were expressed as mean ± standard deviation. Independent sample t-tests were used for comparisons between two groups, and chi-square tests were employed for non-parametric data. A p-value of ≤ 0.05 was considered statistically significant.

III. RESULTS

Demographic Characteristics of HB-Vaccinated Blood Donors

The study included 1,500 blood donors who had received the hepatitis B vaccine during infancy. The average age of the participants was 20.02 ± 1.93 years, with males comprising 61% (916) of the group, and females accounting for 39% (584). The donors had a mean Body Mass Index (BMI) of 25.6 ± 4.05 kg/m². A small portion of the group, 17 donors (1.1%), were married, while the majority, 876 donors (67.2%), came from rural areas (Table 1).

Table 1: Demographic Characteristics of Blood Donors

Characteristic	N (%)	Mean ± SD	Min-Max
Sex			
Male	916 (61%)		
Female	584 (39%)		
Age (years)		20.02 ± 1.93	15-26
15-18 years	364 (24.2%)		
19-21 years	745 (49.6%)		
22-26 years	391 (26%)		
Marital Status			
Single	1,484 (98.9%)		
Married Male	2 (0.1%)		
Married Female	13 (0.86%)		
Divorced Female	1 (0.06%)		
Residence			
Urban	624 (32.7%)		
Rural	876 (67.2%)		
BMI (kg/m²)		25.6 ± 4.05	15.9-58.3
Underweight	22 (1.4%)		
Normal	909 (60.6%)		
Overweight	410 (27.3%)		
Obese	159 (10.6%)		
Education			
High Qualified	946 (63%)		
Middle Certification	88 (5.8%)		
Below Average	414 (27.6%)		
Craftsmanship	52 (3.4%)		

In addition, 4 donors (0.26%) reported having diabetes mellitus and were receiving insulin therapy. A total of 102 participants (6.7%) indicated a history of hospitalization. Of these, 3 donors (0.1%) had a history of intravenous drug use, 3 (0.1%) had previously received blood transfusions, and 2 (0.1%) had tattoos (Table 2).

Table 2: Risk Factors for HBV Infection Among Blood Donors

Factor	N (%)
Diabetes Mellitus	
No	1,496 (99.7%)
Yes	4 (0.26%)
Intravenous Drug Use	
No	1,497 (99.8%)
Yes	3 (0.2%)
Blood Transfusion	
No	1,497 (99.8%)
Yes	3 (0.2%)
Previous Hospitalization	
No	1,398 (93.2%)
Yes	102 (6.8%)
Tattooing	
No	1,498 (99.9%)
Yes	2 (0.1%)
Illegal Sexual Relations	No
Repeated Syringe Use	No
Family History of HBV	No

Protective Anti-HBs Levels Among Vaccinated Blood Donors

Out of the 1,500 blood donors, 501 individuals (33.4%) had anti-HBs titers of 10 mIU/mL or higher, indicating protective immunity. In contrast, 999 donors (66.6%) had titers below this protective level (Table 3).

Table 3: Hepatitis B Surface Antibody Levels among Blood Donors

	Anti-HBs antibody levels (IU/ml)			
	Minimum	**Maximum**	**Mean**	**± SN**
Blood donors n=1500	2	1000	33.1	112.4
Anti-HBs negative blood donor n=999	2	9	3.1	2.12
Anti-HBs positive blood donor n=501	10	1000	93	180.3

The overall mean anti-HBs level for all participants was 33.1 ± 112.4 mIU/mL. For those with titers below 10 mIU/mL, the average was 3.1 ± 2.12 mIU/mL, while those with protective titers had a mean of 93 ± 180.3 mIU/mL. Among the donors who tested positive for anti-HBc, the average anti-HBs titer was 15.75 ± 38.09 mIU/mL. Evaluation of factors such as age, sex, body mass index (BMI), and diabetes mellitus showed no significant correlation with low anti-HBs levels (below 10 mIU/mL) (Table 4)

Table 4: Risk Factors for Loss of Protective Anti-HBs Levels Among Blood Donors

Factor	HBsAb Titer	P-value	Statistical Test
Sex: Male/Female	61.7% / 38.1% vs. 60.6% / 39.3%	0.648 NS	$X^2 = 0.2$
Age (years)	20.01 ± 1.8 vs. 20.06 ± 2.3	0.686 NS	T = 0.4
BMI (kg/m²)	25.4 ± 3.9 vs. 25.7 ± 4.1	0.174 NS	T = 1.35
Marital Status	98.6% / 1.4% vs. 99.1% / 0.9%	0.377 NS	$X^2 = 0.7$
Diabetes Mellitus	100% vs. 99.6% / 0.4%	0.156 NS	$X^2 = 2.01$
Intravenous Drug Use	0% vs. 0.3%	0.219 NS	$X^2 = 1.5$
Previous Hospitalization	6.38% vs. 7%	0.653 NS	$X^2 = 0.2$
Blood Transfusion	0% vs. 0.3%	0.219 NS	$X^2 = 1.5$
Tattooing	0.1% vs. 0.1%	0.618 NS	$X^2 = 0.24$

HBV Infection Among HB-Vaccinated Blood Donors

Of the 1,500 vaccinated blood donors, eight individuals (0.5%) showed signs of prior exposure to hepatitis B, as evidenced by positive anti-HBc test results (Table 5).

Table 5: Hepatitis B Virus Infection Among Vaccinated Blood Donors

Parameter	N (%)
HBsAg	
Negative	1,499 (99.9%)
Positive	1 (0.06%)
HBcAb (Total)	
Negative	1,492 (99.5%)
Positive	8 (0.5%)
HBVDNA (n=8)	
Negative	7 (0.46%)
Positive	1 (0.06%)

The donors with positive anti-HBc had a mean age of 20.3 ± 2.3 years, and six of the eight were male. Among these individuals, seven had anti-HBs levels below 10 mIU/mL, while only one donor had a titer above 10 mIU/mL (110 mIU/mL). One of the donors tested positive for both HBsAg and HBV DNA, indicating an active infection, whereas the other seven who tested positive for anti-HBc were negative for both HBsAg and HBV DNA. An analysis of potential risk factors, including age, gender, marital status, intravenous drug use, hospitalization history, tattooing, and diabetes mellitus, revealed no significant associations with anti-HBc positivity (Table 6).

Table 6. Risk factors for anti-HBc positivity among hepatitis B vaccinated blood donors

		HBcAb		Stat.test	P-Value
		Negative S=1492	Positive S=8		
Sex	Male Female	911 61% 581 39%	5 62.5% 3 37.5%	0.006	0.933 NS
Age (years)	Mean ± SD	20.4 2.2	20.3 2.3	T= 0.41	0.678 NS
BMI (Kg/m^2)	Median IQR	25.2 22.9-27.5	22.9 21.75-24.35	MW= 3154	0.021 NS
Marital status	Single Married	1476 98.9% 16 1.1%	8 100 % 0 0	X^2=0.08	0.768 NS
D.M	No Yes	1488 99.7% 4 0.26%	8 100 % 0 0	X^2=0.021	0.883 NS
HBsAb	Negative Positive	992 66.4% 500 33.5%	7 87.5% 1 12.5%	X^2=0.16	0.208 NS
I.V Take drug		3 0.2%	0 0%	X X^2=0.0.16	0.899 NS
Previous hospitalizations		102 6.83%	0 0%	X^2=0.58	0.443 NS
Blood Transfusion		3 0.2%	0 0%	X^2 *0.016*	0.899 NS
Tattooing		2 0.1%	0 0%	X^2 = *0.01*	0.917 NS

V –DISCUSSION

Over time, the levels of protective anti-HBs antibodies tend to decline after perinatal hepatitis B vaccination, sometimes becoming undetectable. Studies conducted in regions with a high prevalence of HBV have shown that breakthrough infections may occur as this protective immunity wanes [26]. In this study, we observed that 66.6% of blood donors had anti-HBs levels below the protective threshold of 10 mIU/mL, two decades after their initial vaccination. Additionally, 0.46% of these donors, while testing positive for anti-HBc, had undetectable HBV DNA, indicating prior exposure to the virus. We identified a breakthrough HBV infection in 0.06% of the vaccinated blood donors. Previous Egyptian research has also looked at the long-term immunity provided by HB vaccination. One study showed that 67% of healthy Egyptian children maintained protective anti-HBs levels five years post-vaccination [**27**]. Another study by Elrashidy et al. found that approximately 60% of children retained protective antibody levels ten years after their initial vaccination **[28]**. A pooled analysis indicated that 56.39% of Egyptian children aged 1 to 17 years maintained protective anti-HBs levels after vaccination [**29**]. In contrast, our findings show a marked decrease, with only 33.4% of blood donors exhibiting protective levels at a mean age of 20 years. Similarly, in Italy, the percentage of individuals with protective anti-HBs levels dropped to 37% twenty years after primary vaccination [**30**]. This trend suggests that young adults (aged 20 years or older) may be at risk for HBV infection as their immunity declines with age, highlighting the need to evaluate anti-HBs levels in this population.

The rate of anti-HBs protection post-vaccination has been shown to decline more rapidly in certain populations, such as diabetic Egyptian children [**28**]. Meta-analyses suggest that diabetic patients generally exhibit a weaker immune response to the HB vaccine, resulting in lower anti-HBs levels [31]. For instance, diabetic children have been found to have significantly lower protective antibody levels (58.2%) compared to

non-diabetic controls (84%) [**32**]. However, in our study, diabetes mellitus was not identified as a risk factor for reduced anti-HBs levels.

Anti-HBc antibodies indicate an individual's immune response to HBV. The prevalence of anti-HBc among blood donors who were born after the universal HB vaccination program varies, with rates ranging from 1.0% [**33**] to 21.4% [**34, 35**], depending on the age group and region studied. In Gambia, for instance, anti-HBc prevalence among vaccinated individuals increased with age, from 7.9% in those aged 15 to 19, to 20.9% in individuals aged 20 to 24, and to 31.9% in the 25 to 29-year age group [**36**]. This suggests that as anti-HBs levels decline in adolescence and young adulthood, previously vaccinated individuals may no longer be fully protected, potentially leading to HBV exposure and an immune response that includes anti-HBc. In contrast, early childhood studies in Egypt reported anti-HBc positivity rates ranging from 0% to 0.36% [**37, 38**]. Our study found a higher anti-HBc prevalence of 0.5% in donors two decades after vaccination.

Individuals who test negative for HBsAg but positive for anti-HBc are typically considered to have resolved a previous HBV infection. Several studies have documented higher rates of HBV exposure among the general Egyptian population, with anti-HBc rates among unvaccinated blood donors ranging from 7.8% to 14.2% [**39-42**]. Interestingly, our study found a much lower rate of isolated anti-HBc (0.46%) among vaccinated blood donors. In China, the anti-HBc prevalence was significantly lower (1.0%) among those born after the introduction of HB vaccination compared to those born before (15.5%) [**33**]. These findings underscore the importance of HB vaccination in reducing the incidence of HBV transmission and exposure.

Although HBV DNA may not be detectable in patients who are HBsAg-negative and anti-HBc-positive, the virus can persist in the liver as covalently closed circular DNA (cccDNA) [**43**]. Reactivation of HBV can occur in such individuals under certain clinical conditions, such as during chemotherapy or treatment with biologic agents for autoimmune or malignant diseases [**44-49**]. The clinical presentation of HBV reactivation varies, ranging from asymptomatic cases to severe outcomes, including fulminant hepatitis, liver failure, and even death [**50**]. Low or undetectable baseline anti-HBs levels are considered a significant risk factor for reactivation, whereas higher baseline levels offer protection [**51, 52, 53**]. This highlights the importance of assessing baseline anti-HBs levels in HB-vaccinated individuals who are undergoing treatments that may increase their risk of reactivation. HBV infection rates among blood donors have dramatically declined in many countries following the introduction of HB vaccination programs. According to World Health Organization (WHO) data from 2018, the prevalence of HBV among blood donors was 0.02% in high-income countries, 0.29% in upper-middle-income countries, 1.70% in lower-middle-income countries, and 2.81% in low-income countries [**54**]. In Taiwan, the introduction of HBV nucleic acid testing (NAT) revealed that HBV infection rates dropped from 4.53% among donors born before the start of the vaccination program in July 1986 to 0.25% among those born after [**55**]. Similarly, the prevalence of HBsAg among Syrian blood donors was 1.09% among vaccinated individuals, compared to 2.05% among those who were unvaccinated. In Egypt, the overall prevalence of HBsAg among blood donors fell from 2.5–12.7% between 1988 and 1993 [**56**] to 1.8% between 2000 and 2022 [**57**]. In our study, we found an even lower HBsAg rate of 0.07% among blood donors born after the HB vaccination program. A recent meta-analysis reported that Egyptian children under 20 years of age who had been vaccinated had the lowest HBsAg prevalence of 0.69% [**57**]. These results demonstrate the effectiveness of HB vaccination in infancy in providing long-lasting protection and substantially lowering HBV infection rates among blood donors.

Despite the declining HBV exposure and infection rates following widespread vaccination, breakthrough infections still occur among Egyptian children 20 years post-vaccination. Although the rate of chronic HBV carriage remains low, 25% of the anti-HBc-positive donors in our study reported a history of tattooing, though this association was not statistically significant. The observed breakthrough infections may be due to the gradual decline in anti-HBs levels over time. HBsAg prevalence has been shown to increase as the post-vaccination period extends [58]. Specifically, no cases of HBsAg seroconversion were reported within 5 years post-vaccination, but cumulative incidence increased to 0.0006 (2/3422) between 6 and 10 years, and to 0.002 (6/3449) between 11 and 15 years post-vaccination [**58**]. One of our donors with positive anti-HBc and detectable HBV DNA had persistent HBsAg seroconversion. These findings suggest that while three doses of the HB vaccine provide substantial protection for up to 20 years, breakthrough and chronic infections may still occur, underscoring the potential need for booster doses to counter the waning of anti-HBs over time.

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