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Evaluation of Anti-HBs Levels in Children Who Received Three Doses of Hepatitis B Vaccine According to Age Groups and Gestational Age: A Retrospective Single-Center Cross-Sectional Study

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Abstract

Background: Anti-HBs levels decline after infant hepatitis B vaccination, especially during school age and adolescence, but data evaluating both age and gestational age-related effects in a large pediatric cohort remain limited.

Methods: In this retrospective, single-center, cross-sectional study, we included 6,510 children aged 9 months–18 years who had completed three hepatitis B vaccine doses and had at least one quantitative anti-HBs measurement between 2021 and 2023. Seronegativity was defined as anti-HBs <10 mIU/mL. Children were grouped by age (9 months–5 years, 6–11 years, 12–18 years) and gestational age (early preterm <32 weeks, late preterm 32–36^{+6} weeks, term ≥37 weeks). Comparisons used Kruskal–Wallis, chi-square, ANCOVA, and logistic regression.

Results: Overall seronegativity was 30.6% (1,989/6,510), and the median anti-HBs level was 34.30 mIU/mL (IQR 6.72–143.23). Anti-HBs levels decreased significantly with age (medians 99.17, 23.94, and 12.55 mIU/mL for the 9 months–5 years, 6–11 years, and 12–18 years groups; p<0.001), while seronegativity increased (14.4%, 33.9%, and 45.5%; p<0.001). Age group was the strongest independent predictor of seronegativity (6–11 years: OR 3.218, 95% CI 2.521–4.108; 12–18 years: OR 5.348, 95% CI 4.183–6.839). Among 4,242 children with gestational age data, gestational age was not independently associated with seronegativity after adjustment (p=0.109), and seronegativity rates were comparable across groups (34.5% early preterm, 25.8% late preterm, 36.9% term; p=0.168). In children born at term, mean anti-HBs was 525.05±401.31 mIU/mL at ≤3 years versus 74.99±143.60 mIU/mL at ≥4 years.

Conclusion: Anti-HBs levels decline significantly with age after three-dose hepatitis B vaccination, whereas gestational age is not an independent predictor of seronegativity. Targeted serological follow-up and individualized booster strategies, particularly for high-risk groups, may be warranted.

Keywords: Hepatitis B vaccine; anti-HBs; seronegativity; children; gestational age; booster dose

What is known?

- Hepatitis B vaccination is included in national childhood immunization programs, and anti-HBs concentrations decline progressively with age after the primary infant series.
- An anti-HBs concentration ≥10 mIU/mL is conventionally accepted as indicating immunity, but waning — or even undetectable — anti-HBs does not necessarily mean loss of protection, since immunologic memory persists and most vaccine responders still mount a response to a vaccine challenge ≥35 years after vaccination.
- Booster doses are not routinely recommended for immunocompetent children who have completed the three-dose series.

What is new?

- In a large, homogeneous single-center cohort of 6,510 children who had all completed the three-dose (0–1–6 months) hepatitis B vaccine series within the first 6 months of life, median anti-HBs declined

from 99.17 mIU/mL in the 9 months–5 years group to 23.94 mIU/mL at 6–11 years and 12.55 mIU/mL at 12–18 years ($p<0.001$), while seronegativity (<10 mIU/mL) increased from 14.4% to 33.9% and 45.5% (overall 30.6%).

- Despite this decline, both mean and median anti-HBs remained above the protective threshold in all three age groups, indicating that the age-related fall is not by itself a reason for revaccination in healthy children; an exploratory analysis of term-born children further localized the turning point to the fourth year of life (525.05±401.31 mIU/mL at ≤3 years vs. 74.99±143.60 mIU/mL at ≥4 years).
- Gestational age was not an independent predictor of seronegativity (early preterm OR=0.721, 95% CI 0.407–1.277; late preterm OR=0.718, 95% CI 0.403–1.279), but preterm children represented only 121 of the 4,242 children with gestational age data (55 early and 66 late preterm), so the analysis was substantially underpowered.
- The decline therefore warrants targeted serological follow-up in risk groups rather than universal boosting — in children with impaired immune competence, in whom seroconversion after three doses falls to 50–60%, and during adolescence, when exposure-prone behaviors such as piercing, tattooing, and manicure/pedicure performed under inadequate hygiene conditions become more frequent, with injection history rising from 63% at age 13 to 79% at age 17 ($p=0.001$) and piercing/ear piercing from 45% to 55% ($p=0.003$) in a Turkish adolescent cohort.

Introduction

Hepatitis B virus remains a significant public health problem associated with the development of acute and chronic liver disease, cirrhosis, and hepatocellular carcinoma. The World Health Organization emphasizes that one of the most effective strategies for reducing the burden of viral hepatitis is widespread vaccination from birth. Following the implementation of universal newborn hepatitis B vaccination programs, childhood HBsAg prevalence has decreased. Separately, anti-HBs levels decline over time, particularly during school age and adolescence. In countries with a universal newborn vaccination program, anti-HBs levels decline over time, and this becomes more apparent, particularly during the school-age and adolescent periods. Although an anti-HBs level of ≥10 mIU/mL is considered serologically protective, a decrease in antibody levels alone does not always indicate a loss of clinical protection; immunological memory developed after vaccination, especially HBsAg-specific T and B memory cells, can maintain protection even at undetectable antibody levels . Protection for up to 20 years has been reported in fully vaccinated, immunologically competent individuals, and a booster dose is not necessary during this period . Similarly, no increase in chronic HBV has been observed in fully vaccinated cohorts.

In a single-center study from South Korea involving 5,650 children, the mean anti-HBs titer declined continuously until age 4. In a single-center study from South Korea involving 5,650 children, the mean anti-HBs titer declined markedly until age 4, after which it fluctuated. These fluctuations were not statistically significant overall, although a significant decrease was observed between ages 6 and 7. The median titer fell below 10 IU/L in the 7-year-old group, and the lowest mean value (17.73±49.45 IU/L) was observed in the 14-year-old group . experienced another statistically significant decrease between ages 6–7, fell to a median titer of <10 IU/L after age 7, and reached the lowest mean value (17.73±49.45 IU/L) in the 14-year-old group . In a long-term follow-up study, a retrospective cohort study in Beijing, China, covering 30 years post-birth, reported that vaccine efficacy fluctuated and that an adolescent booster administered in junior high school reduced HBV incidence. In a study conducted in Israel, 45.9% anti-HBs positivity was detected 10 years after vaccination and 20.7% at 19 years; the booster dose elicited an anamnestic response in 90.3% of cases. In the cohort born between 1999 and 2016 in the United States, it was emphasized that universal vaccination provided long-term protection in vaccine-related immunity.

Since 1998, hepatitis B vaccination has been part of Turkey's routine childhood immunization schedule. Studies conducted in our country have observed that anti-HBs titers decrease over time and seronegativity rates increase with age . A significant number of studies regarding HBV seroprevalence and vaccine response in the child and adolescent population in Turkey have focused on single-center settings, regionally defined populations, or particular age groups .

In addition, it has been reported that even if passive-active immunoprophylaxis practices with the birth dose of hepatitis B vaccine and hepatitis B immunoglobulin are high in infants born to HBsAg-positive mothers, serological follow-up may remain insufficient under real-life conditions . It is recommended that passive-active immunization be provided together by administering HBV immunoglobulin in addition to the birth dose of the

hepatitis B vaccine in infants of HBsAg-positive mothers . Therefore, individual follow-up and protective titer monitoring in the perinatal period are of great importance in preventing the spread of the disease.

However, prematurity, low birth weight, and immaturity of the immune system are important factors that can affect antibody persistence and the strength of the serological response . It has been reported that in children born preterm, the HBsAb negativity rate at the 5-year follow-up was significantly higher compared to those born at term (47.9% vs. 41.4%, p=0.035; adjusted OR=1.5, 95% CI 1.1–2.0) and that the GMC was lower (9.3 mIU/mL vs. 12.4 mIU/mL, p=0.029) . It was emphasized that preterm infants born at <32 weeks and with a birth weight <2000 g are the priority population for a booster dose . These findings suggest that consideration of an additional HBV vaccine dose should be prioritized for very preterm infants born at <32 weeks of gestation and preterm infants with a birth weight <2000 g, rather than extended to all preterm infants in HBV-endemic regions .

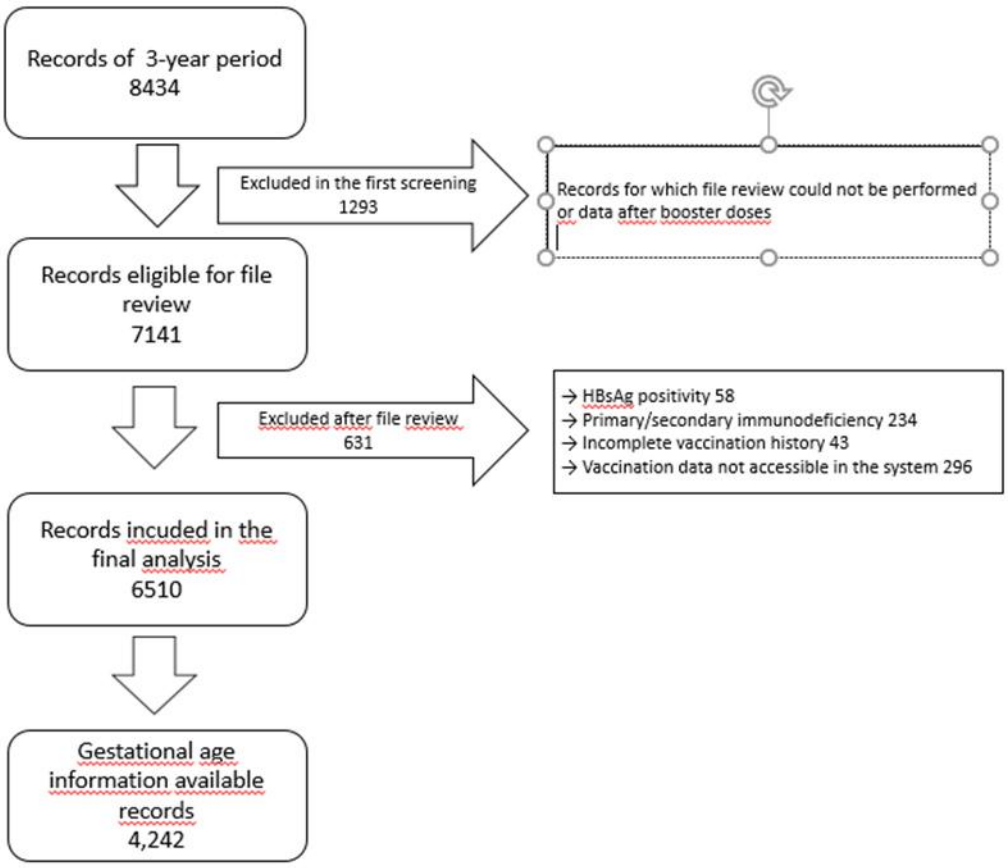
Although previous studies conducted in Turkey have made significant contributions, Although studies from Turkey have described anti-HBs seropositivity in children, much of the available evidence comes from single-center or regionally or age-defined samples . The present retrospective, single-center study examines anti-HBs levels and seronegativity by age and gestational age among children aged 9 months to 18 years who completed three doses of hepatitis B vaccine; gestational-age analyses were restricted to children with available data. To this end, anti-HBs levels were measured at our university hospital over a 3-year period, and the change in serological response with age and its relationship with prematurity in children who received three doses of hepatitis B vaccine were examined comprehensively. The aim of our study is to evaluate anti-HBs levels in a large pediatric cohort based on both age groups and gestational age.

Methods

This study was planned as a retrospective, single-center, cross-sectional, and descriptive analytical study. The data source consisted of the laboratory information system and electronic patient records of Bursa Uludag University Faculty of Medicine Hospital. Ethics committee approval was obtained from the Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee on 05.12.2023 with decision number 2023-25/19 and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki. As the study was retrospective and based on anonymized data extracted from routine medical records, the requirement for written informed consent was waived by the ethics committee.

In the first stage, 8,434 records of patients whose anti-HBs antibody titers were measured at any outpatient clinic of the University Hospital over a 3-year period were identified and evaluated. In the first screening, 1,293 records were excluded: 954 records with no application to the Department of Pediatrics, precluding file review, and 339 control anti-HBs measurements performed after a booster dose or a second vaccination series. After file review, 58 records with HBsAg positivity, 234 with primary/secondary immunodeficiency, 43 with incomplete vaccination history, and 296 whose vaccination data could not be accessed were excluded. When more than one measurement was available within the same age group, the mean of the values was used, whereas measurements from different age groups were retained separately. At the end of this process, 6,510 records were included in the final analysis (Figure 1).

Figure 1. Flow Chart of the Study



Inclusion criteria were being between 9 months and 18 years of age, having completed three doses of the hepatitis B vaccine, and having at least one recorded quantitative anti-HBs measurement. Cases were divided into three groups according to age: 9 months–5 years, 6–11 years, and 12–18 years; and into groups according to birth history: early preterm (<32 weeks), late preterm (32–36+6 weeks), and term (≥37 weeks). Since gestational age data could not be found in the files for all cases, analyses according to gestational age were conducted on 4,242 cases.

At the time of the study, within the scope of the national immunization program in Turkey, hepatitis B vaccination was administered to all children weighing > 2000 g according to the 0-1-6 protocol ; however, since April 2025, with the exception of infants of HBsAg-positive mothers who receive the birth dose, hepatitis B vaccination has been continued as part of the hexavalent (6-component) combined vaccine.

Statistical analysis of the data was performed using the IBM SPSS 29.0.2.0 (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp.) statistical software package. Whether the anti-HBs levels followed a normal distribution was examined with the Shapiro-Wilk test. Descriptive statistics were presented with mean ± standard deviation, median, and 25th–75th percentile values.

Anti-HBs values reported above the upper limit of laboratory measurement (>1000 mIU/mL) were coded as 1000 mIU/mL in the main analysis. Seronegativity was defined as an anti-HBs level below 10 mIU/mL.

Anti-HBs levels were compared between age and gestational age groups using the Kruskal–Wallis test, with pairwise comparisons evaluated by the Bonferroni correction where significant. To exploratively assess a potential age-dependent change pattern in term-born children, those aged ≤3 years were compared with those aged ≥4 years using the Mann–Whitney U test. ANCOVA, adjusted for age and gender, was performed to determine whether anti-HBs levels differed between gestational age groups independently of age at measurement (the anti-HBs assay method had been standardized at our hospital for 20 years, and children who had not completed vaccination within the first 6 months of life were excluded, ensuring a clearly defined interval between the last vaccine dose and antibody measurement), using log10(Anti-HBs+1) as the dependent variable, gestational age as the main independent variable, age as a covariate, and gender as a categorical variable; adjusted pairwise comparisons were reported with the Bonferroni correction. Categorical variables

were presented as frequencies and percentages, and seronegativity rates were compared using the Pearson chi-square test. Logistic regression was used to identify independent factors associated with seronegativity, with anti-HBs <10 mIU/mL as the event group and ≥10 mIU/mL as the reference; age group, gender, and gestational age were included in the model, with 9 months–5 years, male gender, and term birth as reference categories. Results were reported as odds ratios, 95% confidence intervals, and p-values. The interaction between age group and gestational age was evaluated exploratively, but due to low cell frequencies in preterm subgroups and the absence of events in some subgroups, interaction coefficients were not interpreted or included in the main results. $P < 0.05$ was considered statistically significant.

Results

A total of 6,510 children were included in the study. Of the cases, 3,634 were male (55.8%) and 2,876 were female (44.2%). The overall seronegativity rate was found to be 30.6%. The overall median value of anti-HBs levels was 34.30 mIU/mL; the 25th–75th percentile range was determined to be 6.72–143.23 mIU/mL. When evaluated according to age groups, anti-HBs levels decreased with age and seronegativity rates increased (Table 1).

Table 1. Anti-HBs levels, seronegativity, and gender distribution according to age groups

Table 1. Anti-HBs levels, seronegativity, and gender distribution according to age groups

Variable		9 months–5 years n=2,313	6–11 years n=2,173	12–18 years n=2,024	Total n=6,510	p
Anti-HBs,	median (25th-75th)	99,17 (27,21–339,77)	23,94 (5,77–81,46)	12,55 (3,01–52,59)	34,30 (6,72–143,23)	<0,001
	mean±standard deviation	249,02±314,51	88,44±173,58	77,29±178,38	142,03±247,81	
Presence of seronegative	n(%)	332 (%14,4)	736 (%33,9)	921 (%45,5)	1.989 (%30,6)	<0,001
Gender (Female)	n(%)	1.007 (%43,5)	959 (%44,1)	910 (%45,0)	2.876 (%44,2)	0,641

A statistically significant difference was found between age groups in terms of anti-HBs levels (Kruskal–Wallis $H=951.449$; $df=2$; $P < 0.001$). In Bonferroni-corrected pairwise comparisons, there was a significant difference between all age groups. While the median anti-HBs level was 99.17 mIU/mL in the 9 months–5 years group, it declined to 23.94 mIU/mL in the 6–11 years group and to 12.55 mIU/mL in the 12–18 years group. Seronegativity rates also increased significantly with age; the seronegativity rate, which was 14.4% in the 9 months–5 years group, rose to 33.9% in the 6–11 years group and to 45.5% in the 12–18 years group ($\chi^2=510.560$; $df=2$; $P < 0.001$). No statistically significant difference was found between age groups in terms of gender ($\chi^2=0.890$; $df=2$; $P = 0.641$).

The 4,242 children for whom gestational age information was available were evaluated separately. In this group, 55 children were born early preterm, 66 were born late preterm, and 4,121 were born at term. Anti-HBs levels according to gestational age are presented in Table 2.

Table 2. Anti-HBs levels and seronegativity rates according to gestational age

Gestational age	n	Anti-HBs		Seronegative
		mean±standard deviation	median (25th-75th)	n (%)
Early preterm (<32 wk)	55	73,88±188,46	14,84 (5,08–59,63)	19 (%34,5)
Late preterm (32–36 wk)	66	171,32±271,10	47,80 (9,59–174,77)	17 (%25,8)
Term (≥37 wk)	4.121	100,11±197,73	21,57 (4,85–86,15)	1.520 (%36,9)

p	0,033	0,168

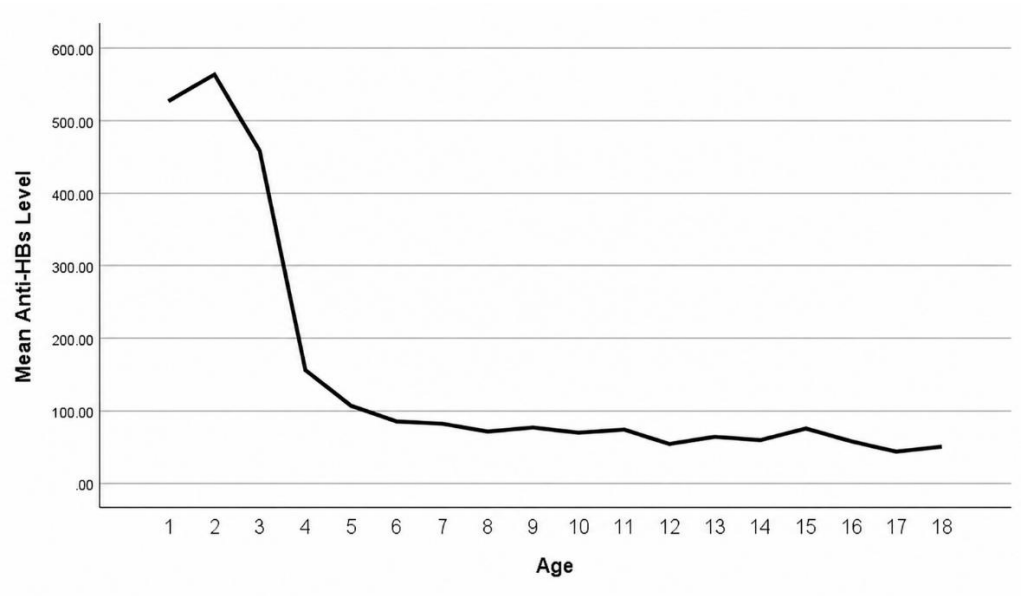
A statistically significant difference was found overall between gestational age groups in terms of anti-HBs levels (Kruskal–Wallis H=6.809; df=2; p=0.033). In Bonferroni-corrected pairwise comparisons, a significant difference was observed only between the late preterm and term groups (p=0.045). No significant difference was found between the early preterm and term groups (p=1.000); the difference between early preterm and late preterm did not reach statistical significance after the Bonferroni correction (p=0.059).

When seronegativity rates were evaluated descriptively between gestational age groups, they were found to be 34.5% in early preterms, 25.8% in late preterms, and 36.9% in those born at term. This finding indicates that there is no distinct and consistent difference between gestational age groups in terms of seronegativity rates (χ^2=3.572; df=2; p=0.168).

Anti-HBs levels between gestational age groups were also evaluated with an analysis adjusted for age of measurement and gender. In the ANCOVA model where the log-transformed anti-HBs level was taken as the dependent variable, age was found to be significantly associated with the anti-HBs level (B=-0.052; p<0.001). In contrast, gender was not significant (p=0.871). After adjustment for age and gender, the overall effect of gestational age on anti-HBs levels did not reach statistical significance; however, a potential effect cannot be excluded given the limited sample sizes in the preterm subgroups(F=2.215; p=0.109). In Bonferroni-corrected pairwise comparisons, no significant difference was found among early preterm, late preterm, and term groups. This finding suggests that the limited differences observed in raw analyses according to gestational age may have been affected by differences in the age of anti-HBs measurement.

The distribution of Anti-HBs levels according to age in children born at term was evaluated exploratively. In the subgroup analysis by age, Anti-HBs levels were higher at age 3 and under and decreased significantly at age 4 and over. Therefore, children born at term were compared in two groups: 3 years and under, and 4 years and over. While the mean Anti-HBs level in children 3 years and under was 525.05±401.31 mIU/mL, it was 74.99±143.60 mIU/mL in children 4 years and over. In Figure 2, the age-related distribution of mean Anti-HBs levels according to gestational age at birth in term individuals is shown. This finding suggests that Anti-HBs levels in children born at term remain high in early childhood and show a significant downward trend at age 4 and after. Therefore, this analysis was interpreted not as a predefined confirmatory threshold analysis, but as an exploratory sub-analysis aimed at evaluating the possible change point dependent on age.

Figure 2. Age-related distribution of mean Anti-HBs levels among children born at term.



Logistic regression analysis was performed on 4,242 children with gestational age data to evaluate independent factors associated with seronegativity. Age group, gender, and gestational age were included in the model. The model was significant overall (χ^2=235.446; p<0.001). Logistic regression results are given in Table 3.

Table 3. Logistic regression analysis of factors associated with seronegativity

Variable	OR	95% CI	p
Age group			
9 months–5 years	Reference	–	–
6–11 years	3,218	2,521–4,108	<0,001
12–18 years	5,348	4,183–6,839	<0,001
Gender			
Male	Reference	–	
Female	1,064	0,935–1,210	0,348
Gestational age			
Term	Reference	–	–
Early preterm	0,721	0,407–1,277	0,262
Late preterm	0,718	0,403–1,279	0,260

OR: odds ratio; CI: confidence interval. The dependent variable is seronegativity, i.e., anti-HBs <10 mIU/mL. Reference categories are 9 months–5 years, male gender, and term birth.

In the logistic regression analysis, age group was found to be independently associated with seronegativity. Compared to the 9 months–5 years group, the probability of seronegativity was approximately 3.22 times higher in the 6–11 years group and approximately 5.35 times higher in the 12–18 years group. In contrast, gender was not significantly associated with seronegativity. Gestational age was also not identified as an independent predictor for seronegativity. No statistically significant difference was found in terms of the probability of seronegativity in early preterm and late preterm groups compared to children born at term.

The interaction between age group and gestational age was evaluated exploratively. However, due to low cell frequencies in preterm subgroups and the absence of events in some subgroups, the coefficients belonging to the interaction model were not interpreted and were not included in the main findings. Current data do not support that the age-dependent increase in seronegativity differs significantly between gestational age groups.

Discussion

This study, which evaluated 6,510 children aged 9 months–18 years who had all completed the three-dose hepatitis B vaccine series within the first 6 months of life, demonstrates a progressive age-related decline in anti-HBs levels accompanied by a parallel increase in seronegativity. Notably, although the median anti-HBs concentration fell from 99.17 mIU/mL in the 9 months–5 years group to 23.94 mIU/mL in the 6–11 years group and 12.55 mIU/mL in the 12–18 years group (p<0.001), both the mean and the median values remained above the 10 mIU/mL threshold accepted as seroprotective in all three age groups, and overall seronegativity was 30.6% (1,989/6,510). This dissociation between waning antibody titers and preserved early protection is the central finding of our work.

Our results are consistent with the existing literatüre (Table-4). Bayhan et al. reported seronegativity rates of 15.2% at 7 months–3 years, 32.8% at 4–7 years, 40.6% at 8–11 years, and 38.6% at 12–14 years among 4,326 Turkish children ; our corresponding values (14.4%, 33.9%, and 45.5%) follow almost the same trajectory, although the adolescent increase was more pronounced in our cohort. In 5,650 South Korean children, Lee et al. observed that mean anti-HBs declined continuously until age 4, plateaued thereafter, decreased significantly at 6–7 years, and fell below a median of 10 IU/L after age 7 . Qawasmi et al. found protective titers in 92.2% of children at age 1, 87.5% at age 2, and 95% at age 4, followed by a decline to 69.2–66.7% at ages 5–6 and an average of 39.8% between 7 and 19 years . Our exploratory analysis in term-born children, showing a mean of 525.05±401.31 mIU/mL at ≤3 years versus 74.99±143.60 mIU/mL at ≥4 years is consistent with this evidence and points to the fourth year of life as a possible kinetic turning point. The same pattern has been documented across Turkish cohorts, with seropositivity declining from 77.8% at 0–1 years to 57.9% at 7–18 years in

Karabük , and from 64.4% to 36.8% after age 7 in Malatya ; our overall positivity of 69.4% (4,521/6,510) closely matches earlier national figures . Collectively, these data indicate that the age-dependent decline is a reproducible biological phenomenon rather than a population-specific artifact.

The clinical interpretation of this decline, however, requires caution. Although anti-HBs ≥10 mIU/mL is conventionally regarded as protective, antibody disappearance does not equate to loss of immunity: HBsAg-specific memory B and T cells can mount a rapid anamnestic response even when anti-HBs becomes undetectable, and concentrations below 10 mIU/mL cannot be interpreted as the absence of protection. Accordingly, the CDC states that although anti-HBs concentrations may wane among vaccine responders, the majority remain immune and respond to a vaccine challenge more than 35 years after vaccination, and that revaccination or booster doses are not routinely recommended for immunocompetent persons . Lee et al. similarly concluded that boosters are unnecessary after a completed three-dose series and that a single dose is sufficient in those who test antibody-negative . In other words, recent evidence indicates that even when the serological threshold is crossed, revaccination is not recommended for individuals who do not belong to a risk group.

Table 4. Literature Review of Anti-HBs Seropositivity Across Different Age Groups Following a Three-Dose Hepatitis B Vaccination Schedule

Reference paper / study design	Study population (N) / overall age range	Age group	Anti-HBs seropositivity, n/N (%)	Statistical analysis
Bayhan et al. (Ref.no 8) Retrospective, single-center July 2011-May 2018	N = 4,326 children who completed three vaccine doses 7 months-18 years Mean age: 127 ± 62 months	7 months-3 years 4-7 years 8-11 years 12-14 years ≥15 years	492/580 (84.8%) 648/965 (67.2%) 450/758 (59.4%) 442/720 (61.4%) 960/1,303 (73.7%)	$p < 0.001$
Song et al. (Ref.20) Prospective follow-up cohort Follow-up: 7 months-5 years	N = 454 children born to HBsAg-positive mothers 7 months-5 years	7 months 1 year 2 years 3 years 5 years	454/454 (100.0%) 445/454 (98.0%) 309/435 (71.0%) 213/326 (65.3%) 183/246 (74.4%)	Not reported
Lee et al. (Ref. 2) Retrospective, single-center 2012-2015	N = 5,650 HBsAg-negative children 0-17 years Mean age: 48.2 months	Overall cohort <2 years 2-4 years >5 years	1,909/5,650 (33.8%) positive; weak-positive: 2,262/5,650 (40.0%)* Highest positivity (>50%) Exact n/N not reported	Inverse association with age; $p < 0.001$
Dogan et al. (Ref. 15) Cross-sectional January 2015-December 2016	N = 2,024 children 0-18 years Mean age: 10.24 ± 4.71 years	Overall cohort 0-1 year 2-6 years 7-18 years	n not reported/2,024 (61.1%) Subgroup n/N not reported (77.8%) Subgroup n/N not reported (71.3%) Subgroup n/N not reported (57.9%)	Not reported
Ozen et al. (Ref. 9) Cross-sectional January 2004-July 2005	N = 989 children 2 months-16 years	2 months-<7 years ≥7 years	≈366/568 (64.4%) ≈155/421 (36.8%)	$p < 0.00001$
Mingir et al. (Ref. 21) School-based cross-sectional prevalence study 2015-2016 academic year	N = 803 students born in 1999 or 2003 13 and 17 years	13 years 17 years	Subgroup n/N not reported (51.6%) Subgroup n/N not reported (90.5%)	$p < 0.001$
Present study Cross-sectional 2021-2023	N = 6,510 children 9 months-18 years	9 months-5 years 6-11 years 12-18 years Overall	1,981/2,313 (85.6%) 1,437/2,173 (66.1%) 1,103/2,024 (54.5%) 4,521/6,510 (69.4%)	Age-group comparison: $p < 0.001$ Seronegativity: $p < 0.001$

				Sex: p = 0.641

** Lee et al. classified results as positive, weak-positive, and negative. The weak-positive category is shown separately because the supplied data did not define whether it should be combined with the positive category for the ≥10 mIU/mL threshold.*

This is precisely where our findings acquire practical relevance. The age-related decline should not be a source of concern in healthy, immunocompetent children, but it warrants attention in children with impaired immune competence or increased exposure risk. Seroconversion after three vaccine doses is approximately 95% in healthy adults but falls to 50–60% in patients with cirrhosis, chronic kidney disease, organ transplantation, celiac disease, immunosuppression, or hemodialysis ; in such groups, anti-HBs levels should be monitored and a booster dose administered when the level drops below 10 mIU/mL, with annual testing recommended for hemodialysis patients . Children born to HBsAg-positive mothers represent a further high-risk group in which the trigger for boosting differs from the serological threshold: in a prospective mother infant cohort of 454 children with successful immunoprophylaxis, 31 (6.83%) experienced vaccine breakthrough infection, and a booster dose given before 2 years of age rather than waiting for anti-HBs to fall below 10 mIU/mL—reduced the incidence of breakthrough infection, with all 14 children who developed infection by age 2 having had anti-HBs >10 mIU/mL at 1 year of age [20]. Adolescence constitutes an additional risk window. Most acute HBV infections occur during adolescence or adulthood and are linked to sexual exposure or injection drug use , and behaviors that create percutaneous exposure opportunities, piercing, tattooing, and personal-care procedures such as manicure and pedicure performed under inadequate hygiene conditions, become increasingly common in this period. In a Turkish adolescent cohort, a history of injection rose from 63% at age 13 to 79% at age 17 (p=0.001) and piercing/ear piercing from 45% to 55% (p=0.003) [21]. The convergence of declining antibody levels with rising behavioral exposure during adolescence therefore justifies targeted serological follow-up in this age group, even though universal boosting is not indicated.

With respect to gestational age, our findings differ from some previous reports. We observed no independent association between prematurity and seronegativity after adjustment for age and gender (early preterm OR=0.721, 95% CI 0.407–1.277; late preterm OR=0.718, 95% CI 0.403–1.279), with comparable seronegativity rates (34.5% early preterm, 25.8% late preterm, 36.9% term; p=0.168). This contrasts with data suggesting that prematurity per se predicts lower anti-HBs concentrations and with reports of higher negativity at 5 years among preterm children (47.9% vs. 41.4%; adjusted OR=1.5, 95% CI 1.1–2.0) and lower geometric mean concentrations (9.3 vs. 12.4 mIU/mL) . Importantly, however, preterm children comprised only 121 of the 4,242 children with gestational age data (55 early and 66 late preterm), so our analysis was substantially underpowered to detect such differences; the null result should therefore be interpreted with caution rather than as evidence against an effect. It is reassuring that almost all preterm infants reach protective concentrations by 9–12 months of age after the recommended three doses .

The principal strengths of our study are the evaluation of a large, homogeneous cohort of 6,510 children who all completed three vaccine doses within the first 6 months of life, the simultaneous consideration of age and gestational age, and the availability of quantitative anti-HBs values in every participant. However, several limitations must be acknowledged. The retrospective, single-center design limits generalizability; gestational age data were unavailable for a substantial proportion of cases, which may have introduced selection bias; and the small preterm subgroups reduced statistical power. Birth weight, maternal HBsAg status, neonatal immunoprophylaxis details, booster history, and immunosuppressive conditions could not be evaluated in detail. Future multicenter, prospective studies incorporating these variables will help clarify the clinical counterpart of the serological decline observed after age 4 and during adolescence.

In conclusion, anti-HBs levels after childhood hepatitis B vaccination decline significantly with age and seronegativity increases, particularly after school age. Prematurity did not emerge as an independent predictor of seronegativity in our cohort, although this finding should be interpreted in light of the small number of preterm children. These results support targeted serological follow-up and individualized revaccination strategies, especially in high-risk groups, rather than universal boosting, and provide real-world evidence to strengthen national pediatric hepatitis B follow-up strategies.

Conclusion

This study shows that anti-HBs levels after childhood hepatitis B vaccination decrease with age and seronegativity increases significantly, especially after school age, with a significant decline around age 4. Prematurity appears to be an important factor affecting serological response strength; however, no significant difference in seronegativity rates by gestational age was found in this limited sample; given the small preterm subgroup, this finding is not conclusive, and larger studies are needed, and it did not emerge as an independent,

linear predictor of seronegativity. These findings suggest that targeted serological follow-up and individualized revaccination strategies, especially in high-risk groups, may be more rational, and future multicenter, prospective studies evaluating serological findings together with cellular immunity and booster response are recommended, thereby strengthening national pediatric hepatitis B follow-up strategies in an evidence-based manner.

Declarations

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Competing interests
The authors declare that they have no competing interests.

Consent to participate
Not applicable. This retrospective study used anonymized data from existing medical records, and the Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee waived the requirement for informed consent (decision number 2023-25/19, dated 05.12.2023).

Consent for publication
Not applicable. This manuscript does not contain data or images from any person in a form that would allow identification.

Clinical trial number
Not applicable. This was a retrospective, single-center, cross-sectional observational study and was therefore not registered in a clinical trial registry.

Data availability
The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
M.K.H. and Z.G.E.Ö. conceived and designed the study and supervised the project. Z.G.E.Ö., F.D.A., E.Ç., S.İ.Ç., and S.Ç.Ç. collected and curated the patient and laboratory data. G.Ö. performed the statistical analysis. M.K.H. and Z.G.E.Ö. wrote the first draft of the manuscript. İ.S., Ş.Ç., S.Ç.Ç., and G.Ö. contributed to the interpretation of the results and commented on previous versions of the manuscript, and M.K.H. and Z.G.E.Ö. revised it critically for important intellectual content. All authors read and approved the final manuscript.

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