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ORIGINAL ARTICLE

Neonatal vaccination and HBV prevalence: evidence from Esfandiar village, Iran

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ABSTRACT

Background and Objectives: Hepatitis B virus (HBV) remains a major public health challenge, particularly in hyperendemic regions. This study assessed the effectiveness of Iran's national HBV vaccination program in Esfandiar village, South Khorasan Province, where HBV prevalence substantially exceeds the national average. We compared hepatitis B surface antigen (HBsAg) prevalence between cohorts born before and after implementation of the universal vaccination program in 1993.
Materials and Methods: We conducted a cross-sectional seroprevalence study encompassing both unvaccinated individuals (born before 1993) and vaccinated individuals (born 1993 onwards) in Esfandiar village. Serum samples were analyzed for HBsAg, hepatitis B e antigen (HBeAg), and hepatitis B core antibody (HBcAb) using enzyme-linked immunosorbent assay (ELISA).
Results: HBsAg prevalence was markedly higher among unvaccinated individuals (22.56%, 132/585) compared to vaccinated individuals (1.19%, 3/252), yielding a vaccine effectiveness of 94.74%. Among vaccinated children, 54% maintained protective antibody titers (>10 mIU/mL), with highest levels observed in children born to HBsAg-positive mothers. Conversely, 46% of vaccinated children demonstrated suboptimal antibody titers (<10 mIU/mL), predominantly among those born to HBsAg-negative mothers. Notably, all three HBsAg-positive vaccinated children were born to mothers with concurrent HBsAg and HBeAg positivity.
Conclusion: The national HBV vaccination program demonstrates remarkable effectiveness in reducing HBsAg prevalence, underscoring the critical importance of universal neonatal immunization in endemic settings. Enhanced preventive strategies, including hepatitis B immunoglobulin (HBIG) administration to infants of HBeAg-positive mothers, could further optimize protection. Sustained surveillance and rigorous adherence to vaccination protocols remain essential for achieving comprehensive HBV control.

Keywords: Hepatitis B; Immunization programs; Infant; Seroepidemiologic studies; Vaccine efficacy

INTRODUCTION

Hepatitis B virus (HBV) is a partially double-stranded DNA virus that specifically targets hepatocytes in humans and non-human primates (1). Upon infection, the virus stimulates excessive production of viral envelope proteins, which subsequently circulate as surface antigens in the bloodstream (2). Transmission occurs primarily through exposure to infected blood and body fluids, including semen and vaginal secretions. In highly endemic regions, mother-to-child transmission and horizontal

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transmission during early childhood represent the predominant routes of infection (3, 4).

The global burden of HBV infection remains substantial. According to the World Health Organization (WHO), approximately 296 million individuals were living with chronic hepatitis B in 2019, with the disease causing an estimated 820,000 deaths annually from complications including liver cirrhosis and hepatocellular carcinoma (5). Chronic HBV infection carries a significant mortality risk, with 15-25% of infected individuals developing fatal liver complications (6).

Although no curative treatment exists for acute hepatitis B infection, antiviral therapies can effectively suppress viral replication in chronic cases, thereby slowing cirrhosis progression, reducing hepatocellular carcinoma incidence, and improving long-term survival outcomes. However, many patients require lifelong treatment to maintain viral suppression (7, 8). Vaccination remains the cornerstone of HBV prevention. The WHO recommends administering the first vaccine dose within 24 hours of birth, followed by two or three additional doses to complete the primary series. Booster doses are typically unnecessary, as vaccine-induced immunity persists for at least 20 years and potentially throughout life (9). For infants born to HBsAg-positive mothers, combining hepatitis B immunoglobulin with vaccination provides optimal protection against perinatal transmission (10).

The Islamic Republic of Iran implemented a comprehensive national HBV vaccination program in 1993, representing one of the earliest large-scale immunization initiatives in the Middle East region. This program mandated the administration of three doses of recombinant hepatitis B surface antigen vaccine to all infants and adolescents (11). This strategic intervention has yielded remarkable results, with national HBV prevalence declining to 1.7% by 2020 (12). However, epidemiological surveillance has identified geographic disparities in disease burden. Notably, Esfandiar village in South Khorasan Province exhibits an HBV prevalence of 16.1%, substantially exceeding the national average (13).

Previous epidemiological investigation in Esfandiar's rural population identified multiple risk factors contributing to this elevated prevalence. Traditional practices such as cupping (hijama), which remains prevalent among the region's gypsy communities and rural residents, alongside informal dental procedures and war-related injuries, constitute significant transmission routes. Furthermore, intrafamilial transmission through household contact and perinatal exposure represents a major contributor to the endemic burden in this community. The persistence of these traditional practices, coupled with limited health literacy, underscores the critical need for culturally appropriate educational interventions and enhanced public health programs tailored to this population's specific needs (13).

This marked regional disparity necessitates a comprehensive evaluation of vaccine effectiveness in high-prevalence areas. Accordingly, the present study aims to assess the impact of Iran's HBV vaccination program in Esfandiar village, with particular emphasis on preventing vertical transmission from hepatitis B surface antigen (HBsAg)-positive mothers to their children. Elucidating these local epidemiological patterns and vaccination outcomes will provide essential evidence for optimizing immunization strategies and reducing transmission in high-burden communities, thereby contributing to the broader success of hepatitis B elimination efforts in Iran.

MATERIALS AND METHODS

Study setting and population. This cross-sectional study was conducted in Esfandiar village, Tabas Golshan city, South Khorasan Province, Iran. The village exhibits an HBV prevalence of 16.1%, substantially exceeding the national average. This endemic setting was selected due to its unique demographic composition, comprising both vaccinated and unvaccinated cohorts. Residents born before 1993 were not included in the national hepatitis B vaccination program, whereas those born from 1993 onward had received the standard three-dose vaccine series as mandated by Iran's national immunization policy. This temporal division provided an ideal natural experiment for evaluating vaccine effectiveness.

Participant recruitment and selection. The study employed a comprehensive sampling approach, recruiting nearly all village residents regardless of age, sex, or residency status. Of 837 individuals assessed for vaccination status, 585 were identified as unvaccinated (born before 1993) and 252 as vaccinated (born 1993 or later). The initial analysis evaluated hepatitis B serological markers in both cohorts to establish baseline prevalence rates.

Subsequently, we identified a subset of 130 unvaccinated mothers from the original cohort who had vaccinated their children (n=201 children total). This mother-child cohort underwent additional analysis to assess vertical transmission patterns and evaluate antibody titers in vaccinated offspring of HBsAg-positive mothers.

Data collection. Demographic information, including maternal age, child age, and sex, was collected through structured questionnaires administered by trained nursing personnel. Written informed consent was obtained from all adult participants and from parents or legal guardians for minors prior to enrollment.

Sample collection. A 5 ml blood sample was taken from each participant. The samples were processed to isolate serum and then analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The analysis focused on key markers associated with hepatitis B infection and immunity, including HBsAg, HBsAb, hepatitis B envelope antigen (HBeAg), and antibodies against hepatitis B core (HBcAb) levels. HBsAg, HBeAg, HBcAb, and HBsAb were measured using the ELISA BIOVANTION kit (Cat. No. TY0031), (Cat. No. BE103A), (Cat. No. BE105A), and (Cat. No. BE102A) respectively.

Vaccine effectiveness (VE) calculation. Vaccine effectiveness was calculated using the screening method formula:

$$VE(\%) = \frac{PPV - PCV}{PPV \times (1 - PCV)} \times 100$$

Where:

- PPV = Proportion of vaccinated individuals in the total population
- PCV = Proportion of vaccinated individuals among HBsAg-positive cases

Data analysis methods. Venous blood samples (5 mL) were collected from each participant using standard phlebotomy techniques. Samples were centrifuged to obtain serum, which was subsequently analyzed for hepatitis B serological markers using enzyme-linked immunosorbent assay (ELISA) methodology. Four key markers were assessed to determine infection status and immunity: hepatitis B surface antigen (HBsAg) was detected using the BIOVANTION ELISA kit (Cat. No. TY0031), hepatitis B surface antibody (HBsAb) was measured using the BIOVANTION ELISA kit (Cat. No. BE102A), hepatitis B envelope antigen (HBeAg) was assessed with the BIOVANTION ELISA kit (Cat. No. BE103A), and hepatitis B core antibody (HBcAb) was evaluated using the BIOVANTION ELISA kit (Cat. No. BE105A). All assays were performed according to manufacturer specifications, with appropriate quality control measures implemented to ensure result reliability and reproducibility.

Ethical considerations. The study protocol was approved by the Ethics Committee of Birjand University of Medical Sciences (approval number: IR.BUMS.REC.1399.457) and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrollment. For participants under 18 years of age, parental or guardian consent was obtained, along with assent from minors when age-appropriate.

RESULTS

Demographic information. Analysis of demographic data revealed a mean maternal age of 41 ± 8.91 years and a mean child age of 13 ± 4.42 years. The study population comprised 96 male and 105 female children (Table 1).

Prevalence of HBsAg, HBeAg, and HBcAb among vaccinated and unvaccinated populations. Among 585 unvaccinated individuals born before 1993 in Esfandiar village, who were excluded from the national HBV vaccination program, 132 (22.56%) tested positive for HBsAg. Within this HBsAg-positive cohort, four individuals demonstrated concurrent HBeAg and HBcAb positivity, whereas the remaining 128 were HBeAg-negative but HBcAb-positive.

In contrast, among 252 individuals born after 1993 who completed the recommended three-dose HBV

Table 1. Demographic characteristics of the study participants

Variable	Category	Mean ± SD / n
Mothers' age (years)	—	41 ± 8.91
Children's age (years)	—	13 ± 4.42
Children's sex	Male	96
	Female	105

vaccination series, only three (1.19%) tested positive for HBsAg. Of these HBsAg-positive vaccinated individuals, two exhibited both HBeAg and HBcAb positivity, while one was HBeAg-negative and HBcAb-positive.

Comparative analysis between vaccinated and unvaccinated cohorts revealed significantly higher prevalences of both HBsAg-positive/HBeAg-negative and HBsAg-negative/HBcAb-positive individuals in the unvaccinated group compared to the vaccinated group ($P < 0.01$) (Fig. 1).

Vaccine effectiveness. Calculation of hepatitis B vaccine effectiveness yielded a rate of approximately 94.74% among the vaccinated individuals.

Maternal HBV status among mothers who had vaccinated their children. Of the 585 unvaccinated individuals, 130 were mothers who had subsequently vaccinated their children. Among these mothers, 27 (20.76%) tested positive for HBsAg, while 103 (79.24%) were HBsAg-negative. Within the HBsAg-positive maternal cohort (n=27), two demonstrated HBeAg positivity and 25 were HBeAg-negative. Among the HBsAg-negative mothers (n=103), 49 tested positive for HBcAb, while 54 were HBcAb-negative (Table 2).

Serological status of children stratified by maternal HBsAg status. Analysis of children born to HBsAg-positive mothers (n=40) revealed three distinct serological profiles: three children were HBsAg-positive, seven were HBsAg-negative/HBcAb-positive, and 30 were negative for both markers. Notably, no children born to HBsAg-negative mothers tested positive for HBsAg (Fig. 2).

HBsAb titers in vaccinated children. Analysis of protective antibody levels demonstrated that children born to mothers with triple positivity (HBsAg+/HBeAg+/HBcAb+) exhibited the highest HBsAb titers (mIU/mL), whereas children born to seronegative mothers (HBsAg-/HBeAg-/HBcAb-) displayed the lowest titers (Fig. 3).

DISCUSSION

In 2016, Ziaee and colleagues conducted a community-based survey to estimate the prevalence of hepatitis B virus (HBV) in the villages of Esfandiar, Marghoub, and Zenuqan in South Khorasan Province, Iran. The results were striking: Esfandiar exhibited a markedly elevated HBV prevalence of 16.1% (13),

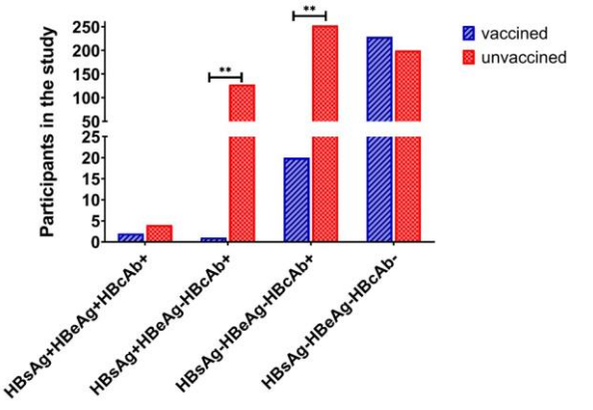

Fig. 1. Prevalence of hepatitis B markers in vaccinated versus unvaccinated populations. Data are presented as mean ± standard deviation. Statistical significance is denoted by * for $P < 0.05$ and ** for $P < 0.01$.

Table 2. Hepatitis B serological profiles of mothers who had vaccinated their children

Maternal HBsAg Status	Subcategory	n	%
Total mothers	—	130	100.00
HBsAg-positive	Total	27	20.76
	HBeAg-positive	2	—
	HBeAg-negative	25	—
HBsAg-negative	Total	103	79.24
	HBcAb-positive	49	—
	HBcAb-negative	54	—

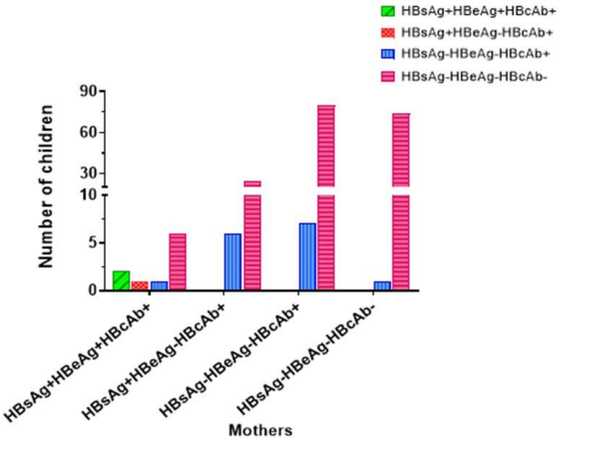

Fig. 2. Hepatitis B serological status of children stratified by maternal HBsAg status.

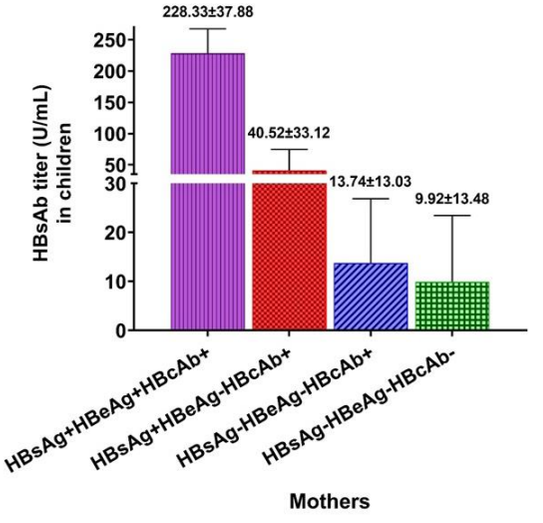

Fig. 3. HBsAb titers (mIU/mL) in vaccinated children according to maternal hepatitis B serological profile.

whereas neighboring villages were below the national average.

Since 1993, children born in Esfandiar have received hepatitis B vaccinations through Iran's national immunization program, consistent with nationwide implementation. To evaluate this program's effectiveness, we examined protection levels among individuals born from 1993 onward.

Our analysis revealed striking differences between vaccination cohorts. Among individuals born before 1993, who were excluded from the vaccination program, HBsAg prevalence reached 22.56% (132 of 585 individuals). Conversely, those born in 1993 or later who had received the complete three-dose HBV vaccine series demonstrated a markedly reduced HBsAg prevalence of only 1.19% (3 of 252 individuals). These findings indicate a vaccine effectiveness rate of approximately 94.74%, underscoring vaccination's critical role in controlling HBV transmission.

Notably, all three vaccinated children who tested HBsAg-positive were born to mothers with both HBsAg and HBeAg positivity, suggesting that elevated maternal viral loads and HBeAg positivity substantially increase perinatal transmission risk. An alternative explanation involves vaccine escape mutants, which may cause infection despite immunization. While vaccination programs have successfully reduced disease prevalence, selective pressure may have facilitated the emergence of mutations that evade antibody-mediated protection. For instance, a glycine-to-arginine substitution at position 145 within the "a determinant" of HBsAg can alter the antigenic structure as well as the immunogenic properties, thereby compromising neutralizing antibodies' recognition and binding capacity (14). Additionally, mutations at positions S120 and S145, frequently identified in cases of immune evasion and vaccine failure, may impair antibody-HBsAg binding, potentially enabling viral immune escape (15).

Our findings align with multiple Iranian studies documenting vaccination program success. Moghadami et al. (2019) demonstrated that incorporating hepatitis B vaccination into Iran's routine immunization schedule significantly has reduced HBsAg carrier rates among individuals born from 1994 onward, with prevalence declining from 3.5% pre-vaccination to 0.6% post-vaccination (12). Similarly, Hashemi et al. reported 67.9% vaccine efficacy among Fars Province students assessed 6-8 years post-vaccination (16). Rezaei et al. (2014) documented protective antibody titers in 88% of children under five years in Semnan Province, with gradual declines to 78% in children aged 5-10 years and 74% at ten years post-vaccination (17). A comprehensive 2018 meta-analysis by Najafi et al. confirmed the vaccine's high efficacy throughout Iran, reporting 89% protection rates in children under five, with slightly higher rates among girls (88%) than boys (85%) (18).

Importantly, the three HBsAg-positive vaccinated children in our study were exclusively born to mothers with both HBsAg and HBeAg positivity; no children born to HBsAg-positive/HBeAg-negative mothers tested HBsAg-positive. Although 15 vaccinated children demonstrated HBcAb positivity, indicating prior viral exposure, none developed acute or chronic hepatitis, further supporting vaccination's protective efficacy. Additionally, 54% of vaccinated children maintained protective antibody titers exceeding 10 mIU/ml, with the highest titers observed among children born to HBsAg-positive mothers, particularly those with concurrent HBeAg positivity. Conversely, 46% of vaccinated children exhibited non-protective titers below 10 mIU/ml, predominantly among those born to HBsAg-negative mothers.

These results demonstrate that maternal HBsAg and HBeAg status significantly influences vaccination outcomes, with infants born to HBsAg-positive—particularly HBeAg-positive—mothers facing elevated infection risk. These findings emphasize the necessity for targeted public health interventions addressing this vulnerable population. Essential strat-

egies include universal maternal HBV screening during pregnancy, timely administration of hepatitis B immune globulin (HBIG) concurrent with birth-dose vaccination for exposed infants, and comprehensive post-vaccination serologic testing to confirm protective immunity. However, implementation barriers in rural and resource-limited settings include restricted HBIG availability, cold-chain maintenance challenges, and financial constraints. Potential solutions encompass strengthening supply chain infrastructure, securing governmental or insurance coverage for HBIG, and integrating maternal HBV screening into standard antenatal care protocols to facilitate prompt identification and treatment of at-risk newborns. Moreover, incorporating systematic follow-up monitoring within existing child health programs enables early identification of non-responders, facilitating timely booster administration or alternative preventive interventions when indicated. Through strategic resource allocation targeting highest-risk children, these approaches can substantially reduce HBV transmission while optimizing national vaccination program effectiveness.

Our study further confirms that while children born to HBsAg-positive mothers require vigilant monitoring, routine booster doses are unnecessary. This finding accords with current WHO guidelines, which recommend against booster vaccination for immunocompetent individuals who have completed the primary series. The observed low antibody titers in some children likely reflect durable immune memory rather than inadequate protection. Collectively, our findings demonstrate the remarkable success of Iran's national hepatitis B vaccination program in reducing HBV transmission, particularly among high-risk children born to HBsAg-positive mothers.

CONCLUSION

Our findings demonstrate that the hepatitis B vaccination program achieves exceptional effectiveness, with a calculated rate of approximately 94.73%. The substantial reduction in HBsAg prevalence among vaccinated individuals underscores the critical importance of neonatal vaccination in HBV-endemic regions. While a limited number of vaccinated individuals born to HBeAg-positive mothers acquired HBV infection, this observation emphasizes the necessity for enhanced preventive strategies targeting this high-risk population, including HBIG administration at birth. Continued surveillance and rigorous adherence to vaccination protocols remain essential for sustaining progress toward comprehensive HBV control.

REFERENCES

1. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; 384: 2053-2063.
2. Elizalde MM, Tadey L, Mammana L, Quarleri JF, Campos RH, Flichman DM. Biological characterization of Hepatitis B virus Genotypes: Their role in viral Replication and antigen Expression. *Front Microbiol* 2021; 12: 758613.
3. Pan CQ, Duan ZP, Bhamidimarri KR, Zou HB, Liang XF, Li J, et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. *Clin Gastroenterol Hepatol* 2012; 10: 452-459.
4. Kidd-Ljunggren K, Holmberg A, Blackberg J, Lindqvist B. High levels of hepatitis B virus DNA in body fluids from chronic carriers. *J Hosp Infect* 2006; 64: 352-357.
5. Zhang C, Liu Y, Zhao H, Wang G. Global Patterns and trends in total Burden of Hepatitis B from 1990 to 2019 and Predictions to 2030. *Clin Epidemiol* 2022; 14: 1519-1533.
6. Ward J, Robinson PJ. How to detect hepatocellular carcinoma in cirrhosis. *Eur Radiol* 2002; 12: 2258-2272.
7. Aspinall EJ, Hawkins G, Fraser A, Hutchinson SJ, Goldberg D. Hepatitis B prevention, diagnosis, treatment and care: a review. *Occup Med (Lond)* 2011; 61: 531-540.
8. Suk-Fong Lok A. Hepatitis B Treatment: What We Know Now and What Remains to Be Researched. *Hepatol Commun* 2018; 3: 8-19.
9. Meireles LC, Marinho RT, Van Damme P. Three decades of hepatitis B control with vaccination. *World J Hepatol* 2015; 7: 2127-2132.
10. Matthews PC, Ocama P, Wang S, El-Sayed M, Turkova A, Ford D, et al. Enhancing interventions for prevention of mother-to-child- transmission of hepatitis B virus. *JHEP Rep* 2023; 5: 100777.
11. Behzadifar M, Azari S, Shirkhani S, Gholamrezaei S, Shahabi S, Doshmangir L, et al. Hepatitis B vaccination in Iran: Historical policies and programs. *J Prev Med Hyg* 2022; 63: E618-E624.
12. Moghadami M, Dadashpour N, Mokhtari AM, Ebrahimi M, Mirahmadizadeh A. The effectiveness of the national hepatitis B vaccination program 25 years after its introduction in Iran: a historical cohort study. *Braz J Infect Dis* 2019; 23: 419-426.

13. Javanmard D, Alavian SM, Abedi F, Namaei MH, Asghari A, Ziaee M. High prevalence of hepatitis B virus infection in the Village of Esfandiar in South Khorasan Province, Iran. *Hepatol Mon* 2018; 18(8): e65473.
14. Leong J, Lin D, Nguyen MH. Hepatitis B surface antigen escape mutations: Indications for initiation of antiviral therapy revisited. *World J Clin Cases* 2016; 4: 71-75.
15. Li S, Xie M, Li W, Peng Q, Zhong B, Lu X, et al. Prevalence of S gene mutations within the major hydrophilic region of hepatitis B virus in patients in Dongguan, southern China. *Arch Virol* 2017; 162: 2949-2957.
16. Hashemi SA, Moghadami M, Lankarani KB, Alborzi A, Mahboudi A. The efficacy of hepatitis B vaccination among school age children in Southern Iran. *Iran Red Crescent Med J* 2010; 12: 45-48.
17. Rezaei M, Nooripoor S, Ghorbani R, Ramezanshams F, Mamishi S, Mahmoudi S. Seroprotection after hepatitis B vaccination in children aged 1 to 15 years in central province of Iran, Semnan. *J Prev Med Hyg* 2014; 55: 1-3.
18. Najafi F, Sayehmiri K, Najafi R. Efficacy of hepatitis B vaccination in under five-year-old children in Iran: A systematic review and meta-analysis study. *Hepat Mon* 2018;18(6): e65385.