Evaluation of Factors Affecting the Expression of Anti-HBs in Children in Hunan Province

Shan Tan

Central South University Third Xiangya Hospital

Shizhou Li

Central South University Xiangya School of Medicine

Jianxiang Dong

Central South University Xiangya School of Medicine

Hongmei Dai

Central South University Third Xiangya Hospital

Minghua Yang

Central South University Third Xiangya Hospital

Fang Sun

Central South University Third Xiangya Hospital

Yang Yang

Central South University Xiangya School of Medicine

Jie Jiang

Central South University Third Xiangya Hospital

Mingyi Zhao (✉ zhao_mingyi@csu.edu.cn)

Central South University Third Xiangya Hospital

Research Article

Keywords: HBV (hepatitis B virus), GMT (geometric mean titer), BMI (body mass index)

Posted Date: October 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-962347/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background and objectives

Vaccine is the most essential avenue to prevent hepatitis B infection in infants and preschool children in China, with the largest populations carrying hepatitis B virus in the world. This study aimed to evaluate the factors affecting the response level of anti-HBs in children, with a view to provide instructions for hepatitis B prevention clinically.

Methods

The children taking physical examinations in the Third Xiangya Hospital from January 2013 to April 2020 were recruited. Telephone follow-up were adopted to collect further information. Univariate logistic regression was used to analyse the relationship between age and anti-HBs expression. After grouping by age and anti-HBs expression, we used chi-square test and T test to compare the difference between positive and negative antibody expression in each age subgroup. The meaningful variables ($P < 0.10$) in chi-square test and T test were assessed with collinearity and chosen for univariate and multivariate logistic regression analysis by the stepwise backward maximum likelihood method ($\alpha_{in} = 0.05$, $\alpha_{out} = 0.10$).

Results

A total of 5838 samples (3362 males, 57.6%) were enrolled. The outcomes showed that the expression of anti-HBs was associated with age [OR=1.037(1.022-1.051)] in whole sample. Anemia[OR=0.392(0.185-0.835)], age[OR=2.542(1.961-3.295)] and Vit D[OR=0.977(0.969-0.984)] in 0.5-2.99 years subgroup, Zinc deficiency[OR=0.713(0.551-0.923)] and age[OR=1.151(1.028-1.289)] in 3-5.99 years subgroup, Vit D[OR=0.983(0.971-0.995)] in 12-18 years subgroup had significant effect on the expression of anti-HBs.

Conclusions

This retrospective study illustrated that age, anaemia status, zinc deficiency and vitamin D were associated with the expression of anti-HBs in different age groups children, which could serve as a reference for the prevention of hepatitis.

Summary

Table of Contents Summary: This retrospective study illustrated that age, anaemia status, Zinc and vitamin D were independently associated with the expression of anti-HBs in children.

What's Known on This Subject: Vaccine is the most essential avenue to prevent hepatitis B in infants and preschool children in China which is affected by multiple factors such as geographic location and immunization scheme.

What This Study Adds: Age, anaemia status, zinc and vitamin D are independently associated with the expression of anti-HBs in children, which could serve as a reference for the prevention of hepatitis.

Introduction

Hepatitis B virus infection has been a worldwide sanitary problem threatening individuals' health quality. According to a report of World Health Organization, in 2015, the global prevalence of HBV infection in the general population was estimated at 3.5%, nearly 257 million people suffered from chronic HBV infection worldwide and estimated 887,220 persons died of HBV infection[1]. China had the most citizens carrying HBV. In a study conducted during 2013-2017, the prevalence of HBV infection in the general population of China from 2013 to 2017 was 6.89% (95% CI, 5.84–7.95%), and predicted there were 83,864,139 (95% CI: 60,406,793-110,751,614) infected case in 2018[2].

HBV transmission happening at the perinatal period and early childhood is an important pattern of transmission[3]. To prevent HBV infection of infants and preschool children and control HBV infection in China, it's essential to increase the proportion of children vaccinated hepatitis B vaccine[4]. In 2002, the Chinese authority implemented a new vaccine strategy in which all vaccines, including the hepatitis B vaccine listed in China's National Immunization Programme, were offered freely to neonates and infants. After this, the hepatitis B vaccine coverage rate increased significantly and the HBsAg prevalence also decreased in China. Among the distinct age groups, HBV prevalence in children younger than 15 years old was lower than 2% as a result of the HBV immunization scheme for infants[2]. The results of an epidemiological survey showed that HBsAg prevalence declined from 10.5% to 0.8% among children <15 years and 9.9% to 0.3% among children <5 years during 1992-2014[5].

However, existing studies have revealed that there are various factors that affected the response level to immunization. For instance, the geographic location had a perceptible influence on the efficiency of vaccines implemented in infants. The risk of a low-level response to immunization in rural areas was 2.093 times higher than that in cities[6].

Several studies have evaluated the tangible ability to maintain the level of anti-HBs of the classical pattern of HBV immunization where individuals are injected with the vaccine at 0, 1, and 6 months after birth. First, the time to completion of vaccination is the primary factor. Xiaomei Yue followed the serum anti-HBs levels of Chinese children who received the hepatitis B vaccine as planned. The positive rates of serum anti-HBs (≥ 10 mIU/ml) were 16190/17928(90.31%) for 1 age group, 10755/12808(83.95%) for 2 age group, 6855/9543(71.82%) for 3 age group, 4997/8042(62.14%) for 4 age group[7]. In another study, positive rates of serum anti-HBs for 1-4 years Chinese children were 79/83(95.3%) for 1 age group, 47/53(88.2%) for 2 age group, 44/61(72.1%) for 3 age group, 82/141(58.2%) for 4 age group[8]. The results of the above two studies showed that the positive rate of anti-HBs decreased rapidly during the period from 1 to 3 years old and gradually stabilized after about 4 years old. In addition, the status of maternal immunization[9], large for gestational age[10], dosing schedule[11], vaccine dosage[12] and mother's educational background[13]. However, there is still an immense absence of more objective

factors influencing the response level associated with the individuals themselves, such as their nutrition condition and the method of birth.

Studies in adults have tested other variables that may affect immune status among those receiving vaccines. Variables have been determined to decrease response levels include the site of injection (the gluteal site of injection decreases its efficiency)[14], obesity[15, 16], cigarette smoking, older age[14-16] and the presence of diseases that alter the immune system[16]. No previous studies have conducted a systematic review targeting these variables in children.

In this paper, we evaluate the influence of multiple factors ranging from the detailed objective biochemistry index value to different avenues of birth and feeding on the immunization level of hepatitis B vaccine among children. We also sought to determine the potential co-relationship between different factors affecting that relationship, and provide instruction for hepatitis B prevention clinically.

Methods

Participant recruitment

Children between 6 months and 18 years old attending physical examinations at the third Xiangya Hospital of Central South University, Changsha, Hunan, during January 2013 and April 2020, were recruited in this study (n=100997). Firstly, we eliminated participants who did not undergo anti-HBs detections (n=56181) or defined as positive in HBsAg test (n=57) before further analysis. Thereafter, we established precise inclusion criteria for the remaining candidates having negative HBsAg (n=44759) to generate valid subjects. Herein, participants lacking of essential information such as Gender, Age, way of birth, BMI, hemoglobin, Lead, Cadmium, Zinc, Copper, Calcium, Iron, WBC, VitD, C3, C4, IgA, IgG, IgE, IgM (n=9180) were not eligible. After which, individuals who were low birth weight (<2500 g, n=242), premature (<37 weeks, n=430), combined with diseases which might effect the response level of immunization, such as organic disease related to heart, lung, liver and kidney, allergic disease including asthma, anaphylactoid purpura and other immunological diseases (n=556) were excluded. Next, we filtered subjects failing to follow the obligational hepatitis B vaccination plan (n=10887), where children would be vaccinated within 24 hours, 1 month and 6 months (n=10887) as well as having revaccination before this physical examination (n=15150). Then we obtained further information of mentioned participants (n=8314), including duration of lactation period, history of eczema, frequency of cold and exposure to second-hand cigarette smoke by telephone follow-up. Herein, subjects who were unable to provide precise and complete data ascribed to memory bias or loss to follow-up (n=1206) were eliminated. Then we excluded participants whose mother had ever been infected with hepatitis B before delivery or given hepatitis B immune globulin at birth (n=1270) before. The final candidates (n=5838) were divided into an anti-HBs-positive group (n=3824) and an anti-HBs-negative group (n=2014) (Figure 1). This research was approved by the ethics committee of the third Xiangya Hospital of Central South University and in accordance with medical ethics. Parental consent in person was achieved for participants under 18 years old.

Statistics reference

The references for statistics were illustrated in Table 1 and Table 2, including status of anemia, body mass index, level of microelements, such as, Cu, Ca, Mg, Fe, Zn, Pb, Cd and the classification standard for division of subjects referring to the amount of anti-HBs. Because there is no unified BMI standard for children under 6 years old, we don't discuss it in this paper. We applied the standard of inhalation of environmental tobacco smoke as the definition of being exposed to SHS[17] and more than 6 colds per year as a definition of frequent colds.

Table 1

The statistic reference of indicators.

Indicator	statistic reference
Anemia	
7~59 months old	<110 g/L
5~11 years old	<115 g/L
12~14 years old	<120 g/L
Standards of microelements	
Cu	11.8~39.3 μmol/L
Ca	1.55~2.10 mmol/L
Mg	1.12~2.06 mmol/L
Fe	7.52~11.82 mmol/L
Zn	
0~0.99 years old	58~100 μmol/L
1~1.99 years old	62~110 μmol/L
2~2.99 years old	66~120 μmol/L
3~4.99 years old	72~130 μmol/L
≥5 years old	76.5~150 μmol/L
Pb	0~100 μg/L
Cd	0~5 μg/L
Intoxication levels	
Pb	>100 μg/L
Cd	>2.5 μg/L
Anti-HBs	
Positive	>10mIU/mL
Negative	≤10mIU/mL

Table 2

The statistic reference of BMI.

Age	Normal BMI range	
	Male	Female
6~6.99 years	13.1-17	12.7~17.3
7~7.99 years	13.3~17.4	12.9~17.7
8~8.99 years	13.5-18	13.1~18.4
9~9.99 years	13.7~18.5	13.5~19
10~10.99 years	14~19.2	13.9~19.9
11~11.99 years	14.5~20	14.5~20.8
12~12.99 years	15~20.9	15~21.8
13~13.99 years	15.5~21.7	15.5~22.7
14~14.99 years	16~22.4	16.2~23
15~15.99 years	16.8~23	17.1~23.4
16~16.99 years	17.5~23.5	17.6~23.7
17~18 years	18~24	18~24

The children whose BMI was lower than the reference range were considered as thin, while BMI was higher than reference range were considered as overweight.

Statistical analysis

Enumeration data were described by n (%). Measurement data meeting the criterion of a normal distribution were showed by means and standard deviations. All variables were processed by univariate logistic regression analysis, using odds ratios (ORs) and 95% confidence intervals (CIs) to assess the extent of the relevance. The variables which P value < 0.10 in the chi-square test and T test in any age subgroup were selected for univariate logistic regression analysis, then the variables which P value < 0.10 were selected for further multivariate logistic regression analysis for each age subgroup separately. All data were analysed through SPSS (IBM SPSS Statistics for Macintosh, Version 24.0, Armonk, NY: IBM Corp). P < 0.05 was considered statistically significant.

Results

A total of 5838 children were enrolled in this study, including 2476 (42.4%) girls and 3362 (57.6%) boys, among whom there were 2014 (34.5%) anti-HBs-negative children and 3824 (65.5%) anti-HBs-positive children. As mentioned earlier, time to vaccination has a significant impact on vaccine response rates. Therefore, we adopt the univariable logistics regression to analyse the relationship between age and anti-HBs expression. In the whole samples, for each additional year of age, the probability of anti-HBs

negative increased by 3.7%[OR=1.037(1.022-1.051)]. Since age has a great influence on anti-HBs expression, we divided the samples into four subgroups according to the characteristics of children's development. 0.5-2.99 years subgroup have 1166 samples, among them 913(78.3%) samples were anti-HBs positive. 3-5.99 years subgroup have 1680 samples, among them 1025(61%) samples were anti-HBs positive. 6-11.99 years subgroup have 2265 samples, among them 1407(61.8%) samples were anti-HBs positive. 12-18 years subgroup have 727 samples, among them 479(65.9%) samples were anti-HBs positive.

Each age subgroups were further divided into anti-HBs positive group and negative group. Chi-square test was used to analyze differences of inenumeration data and T test was used to analyze differences of measurement data conforming to normal distribution between anti-HBs positive group and negative group. As the result shows in Table 3 and Table 4, in the 0.5-2.99 years subgroup, anemia($P<0.001$), age($P<0.001$), WBC($P=0.011$) and Vit D($P<0.001$); in the 3-5.99 years subgroup, zinc deficiency($P=0.022$) and age($P=0.014$); in the 12-18 years subgroup, Vit D($P=0.012$) have significant difference between anti-HBs positive and anti-HBs negative group.

Subsequently, variables with $P<0.1$ in chi-square and T test were included in the univariable logistics regression analysis. In 0.5-2.99 years subgroup, anemia [OR=0.275(0.132-0.573), $P<0.001$], WBC[OR=0.93(0.872-0.992), $P=0.028$] and VitD [OR=0.978(0.971-0.986), $P<0.001$] were protective factor for anti-HBs expression, while age [OR=2.476(1.932-3.172), $P<0.001$] was risk factor. The probability of anti-HBs negative increased by 147.6% for each age. In 3-5.99 years subgroup, sufficient zinc [OR=0.713(0.551-0.923, $P=0.01$)] was protective factor for anti-HBs expression, while age [OR=1.151(1.028-1.289), $P=0.014$] was risk factor. In 6-11.99 years subgroup, no statistically significant influencing factors were obtained. In 12-18 years subgroup, VitD [OR=0.984(0.972-0.996), $P=0.01$] was protect factor for anti-HBs expression (Table 5, Figure 2). The OR of age, WBC, VitD means each additional unit of these variables increases the probability of anti-HBs negative for $(OR-1)\times 100\%$.

Then, variables with $P<0.1$ in univariable logistics regression analysis were included into multivariable logistics regression. Use stepwise backward maximum likelihood method to eliminate meaningless variables ($\alpha_{in} = 0.05$, $\alpha_{out} = 0.10$). Except that WBC was excluded, the results were similar to those of univariate logistic (Table 6, Figure 3).

Table 6
Multivariable logistic regression analyze results of four age subgroups.

Variable	OR(95%CI)	P value
0.5~2.99 years(n=1166)		
Anemia		
No	0.392(0.185-0.835)	0.015**
Yes#		
Cadmium intoxication		
No	1.88(0.93-3.802)	0.079*
Yes#		
Age	2.542(1.961-3.295)	<0.001***
VitD(ng/ml)	0.977(0.969-0.984)	<0.001***
3~5.99 years(n=1680)		
Zinc deficiency		
No	0.713(0.551-0.923)	0.01**
Yes#		
Copper deficiency		
No	1.264(0.998-1.6)	0.052*
Yes#		
Age	1.151(1.028-1.289)	0.015**
6~11.99 years(n=2265)		
Whether the children had a history of second-hand cigarette exposure		
No	0.841(0.697-1.015)	0.072*
Yes#		
Zinc deficiency		

#Reference group; *P<0.10; **P<0.05; ***P<0.01.

Variable	OR(95%CI)	P value
No	0.84(0.695-1.016)	0.073*
Yes#		
12~17.99 years(n=727)		
Zinc deficiency		
No	1.355(0.991-1.855)	0.057*
Yes#		
VitD(ng/ml)	0.983(0.971-0.995)	0.007***
#Reference group; *P<0.10; **P<0.05; ***P<0.01.		

Discussion

This research studied multiple factors that may affect the response level of hepatitis B immunization, ranging from exterior elements such as the way of birth and duration of the lactation period to biochemical factors in the children. The outcome of our study demonstrated that age or time since vaccination remains a major factor affecting anti-HBs expression, which is consistent with previous study[7, 8]. In 0.5-2.99 years subgroup and 3-5.99 years subgroup, age significantly affected the expression of anti-HBs, while the 6-11.99 years subgroup and 12-18 years subgroup didn't.

In this report, we found anaemia was able to statistically increase the anti-HBs level in 0.5-2.99 years subgroup. However, there are still controversial opinions about the immune response in anaemia patients. An American study showed that the prevalence of iron deficiency (ID), anemia, and iron deficiency anemia (IDA) among children 1–2 years (12-35.9 months) was 13.5% (9.8, 17.2), 5.4% (3.5, 7.4), and 2.7% (1.2, 4.2) respectively[18]. This shows that anemia is a common child health problem. Continued breastfeeding may increase IDA prevalence in 6-to-23 months children, and the associations of anaemia with inflammation, zinc deficiency and infections could be suggesting the occurrence of nutritional immunity[19]. A study of Kenyan infants showed that correction of iron deficiency may improve response of diphtheria, pertussis, and pneumococcal vaccines during early infancy[20]. Further research is needed on the effect of anemia on the response of hepatitis B vaccination.

A review of Vit D mentioned that Vit D might have a beneficial impact on the body's immune system and modulate both innate and adaptive immunity[21], which is also supported by our study, where the anti-HBs positively rate corresponded with the level of Vit D in vivo. A clinical study showed that serum concentrations of 25(OH)D effect the level of IL-6, TNF- α , and CRP. Children with status ≥ 75 nmol/L had

high level of IL-6, TNF- α , and CRP compared to the <50 nmol/L and 50–74.9 nmol/L categories[22]. Based on these results, 75nmol/L is recommended as an appropriate vitamin D serum concentration. Another notable fact is that vitamin D was statistically significant in 0.5-2.99 years subgroup and 12-18 years subgroup. These two ages subgroup correspond to the two most rapid growth periods for children – infancy and adolescence. However, to reach a more absolute conclusion about the function of Vit D in the immune system, more studies need to be performed on its molecular mechanisms.

Zinc is essential for cellular processes in all cells, especial for cells with a rapid rate of turnover such as those of the immune, gastrointestinal systems, and skin. These cells are particularly vulnerable to zinc deficiency[23]. Interestingly, this effect was only statistically significant in the 3-5.99 subgroup in our research. The effect of zinc on vaccination remains to be further studied.

In contrast with studies aimed at adults[14], no significant difference was confirmed between immune response and second-hand cigarette exposure. In fact, the detrimental outcome of cigarettes on immunologic reaction demands long-term exposure to smoke. Children are exposed to less smoke for a shorter time, making the adverse effect of smoking more formidable to discover.

Conclusion

This study listed possible factors that might influence the response level to hepatitis B vaccines, providing a reference for both parents and health policy-makers. In the domain of efficient prevention of hepatitis, we recommended that parents should provide their children with a healthy and balanced nutrition and sufficient Vit D, Zinc to maintain the quantity of antibodies. In addition, enhance the education of HBV infected mothers is a great way for the prevention of hepatitis B in children. Although this study found that children with anemia in the 0.5-2.99 subgroup were more possible to be anti-HBs positive, there is no clear evidence that this is beneficial for the long-term maintenance of anti-HBs. We recommend that children with anaemia should receive appropriate treatment as soon as possible and recover from anaemia.

The limitation of this research

The methodology of this study was similar to a case-control study, so there is a certain lag in the observation of the outcome (the time of negative anti-HBs appearance). Older children may turn anti-HBs negative prior to the test. For example, a 12-year-old with negative antibodies is likely to have turned negative by age 4 and continued. Therefore, we cannot determine the exact time when their antibodies turn negative. The study did not distinguish between anti-HBs positive groups(≥ 10 mIU/ml) and anti-HBs high response group(≥ 100 mIU/ml). In additional, the subjects of the research were selected from the participants participating in voluntary physical examinations in Hunan Province. Consequently, there might be some meaningful patients omitted, leading to bias to some extent. Although explicit inclusion criteria were applied, there was still unavoidable memory bias of subtle details when completing the telephone follow-up survey.

Declarations

Acknowledgements

None

Funding/Support:

None.

Availability of data and materials

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

Authors' contributions

Shan Tan and Jianxiang Dong conceptualized and designed the study, and reviewed and revised the manuscript.

Shizhou Li drafted the initial manuscript, and reviewed and revised the manuscript.

Hongmei Dai and Minghua Yang designed the data collection instruments, collected data, carried out the initial data analysis, and reviewed and revised the manuscript.

Fang Sun and Jie Jiang made the figure and tables, and reviewed and revised the manuscript.

Mingyi Zhao conceptualized and designed the study, coordinated, and supervised data collection, and critically reviewed the manuscript for important intellectually content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of work.

Ethics approval and consent to participate

This study protocol was approved by the Ethics Committee of The Third Xiangya Hospital of Central South University. Written consent from participants or their primary caregiver was not necessary for this study, as it involved accessing medical records to retrospectively obtain children's gender, age, and laboratory findings. During the telephone follow-up, we explained the purpose of the investigation, and obtain the consent of the respondents orally.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Role of Funder/Sponsor (if any):

The funder/sponsor did not participate in this work.

References

1. Hepatitis B vaccines: WHO position paper - July 2017. *Wkly Epidemiol Rec*, 2017. 92(27): p. 369-92.
2. Wang, H., et al., Hepatitis B infection in the general population of China: a systematic review and meta-analysis. *BMC Infectious Diseases*, 2019. 19(1).
3. Yao, G.B., Importance of perinatal versus horizontal transmission of hepatitis B virus infection in China. *Gut*, 1996. 38(Suppl 2): p. S39-S42.
4. Zhou, Y.H., C. Wu and H. Zhuang, Vaccination against hepatitis B: the Chinese experience. *Chin Med J (Engl)*, 2009. 122(1): p. 98–102.
5. Cui, F., et al., Prevention of Chronic Hepatitis B after 3 Decades of Escalating Vaccination Policy, China. *Emerging Infectious Diseases*, 2017. 23(5): p. 765–772.
6. Zhou SY, D.H.B.G., Non-and-low response after hepatitis B vaccine of 1085 infants in Ningbo and analysis on its influential factors. *Zhonghua Liu Xing Bing Xue Za Zhi*, 2011. 32(9): p. 951–2.
7. Yue, X., et al., Changes and analysis of anti-HBs titres after primary immunization in 1- to 16-year-old Chinese children: A hospital-based study. *Journal of Viral Hepatitis*, 2018. 25(4): p. 373–380.
8. He, F., et al., The Serum Anti-HBs Level Among Children Who Received Routine Hepatitis B Vaccination During Infancy in Mianyang City, China: A Cross-Sectional Study. *Viral Immunology*, 2016. 29(1): p. 40–48.
9. Zhang, W., et al., Maternal immunization promotes the immune response of neonates towards hepatitis B vaccine. *Journal of Viral Hepatitis*, 2013. 20(12): p. 875–881.
10. Çekmez, F., et al., Response to hepatitis B vaccine differs by birthweight among neonates. *Vaccine*, 2011. 29(17): p. 3096–3097.
11. Middleman, A.B., et al., The effect of late doses on the achievement of seroprotection and antibody titer levels with hepatitis b immunization among adolescents. *Pediatrics (Evanston)*, 2001. 107(5): p. 1065.

12. Kang, G., et al., Efficacy of antigen dosage on the hepatitis B vaccine response in infants born to hepatitis B-uninfected and hepatitis B-infected mothers. *Vaccine*, 2015. 33(33): p. 4093–4099.
13. Ertekin, V. and M.A. Selimoglu, Effects of several socio-demographic factors on hepatitis B immunization rates. *European Journal of Gastroenterology & Hepatology*, 2004. 16(7): p. 719.
14. Shaw, F.J., et al., Effect of anatomic injection site, age and smoking on the immune response to hepatitis B vaccination. *Vaccine*, 1989. 7(5): p. 425–30.
15. Roome AJ, W.S.C.M., Hepatitis B Vaccine Responsiveness in Connecticut Public Safety Personnel. *JAMA*, 1993. 270(24): p. 2931–2934.
16. Hollinger, F.B., Factors influencing the immune response to hepatitis B vaccine, booster dose guidelines, and vaccine protocol recommendations. *The American journal of medicine*, 1989. 87(3A): p. 36S.
17. Davey, S.G., Effect of passive smoking on health. *BMJ*, 2003. 326(7398): p. 1048–9.
18. Gupta, P., et al., Iron, Anemia, and Iron Deficiency Anemia among Young Children in the United States. *Nutrients*, 2016. 8(6): p. 330.
19. Fançony, C., et al., Iron deficiency anaemia among 6-to-36-month children from northern Angola. *BMC Pediatrics*, 2020. 20(1).
20. Stoffel, N.U., et al., Iron Deficiency Anemia at Time of Vaccination Predicts Decreased Vaccine Response and Iron Supplementation at Time of Vaccination Increases Humoral Vaccine Response: A Birth Cohort Study and a Randomized Trial Follow-Up Study in Kenyan Infants. *Frontiers in Immunology*, 2020. 11.
21. Mailhot, G. and J.H. White, Vitamin D and Immunity in Infants and Children. *Nutrients*, 2020. 12(5): p. 1233.
22. Brett, N., et al., Vitamin D Status and Immune Health Outcomes in a Cross-Sectional Study and a Randomized Trial of Healthy Young Children. *Nutrients*, 2018. 10(6): p. 680.
23. Ackland, M.L. and A.A. Michalczyk, Zinc and infant nutrition. *Archives of biochemistry and biophysics*, 2016. 611: p. 51–57.

Tables

Due to technical limitations, tables 3, 4 and 5 are only available as a download in the Supplemental Files section.

Figures

Figure 1

Then we excluded participants whose mother had ever been infected with hepatitis B before delivery or given hepatitis B immune globulin at birth (n=1270) before. The final candidates (n=5838) were divided into an anti-HBs-positive group (n=3824) and an anti-HBs-negative group (n=2014) (Figure 1).

Figure 2

In 3-5.99 years subgroup, sufficient zinc [OR=0.713(0.551-0.923, $P=0.01$)] was protective factor for anti-HBs expression, while age [OR=1.151(1.028-1.289), $P=0.014$] was risk factor. In 6-11.99 years subgroup, no statistically significant influencing factors were obtained. In 12-18 years subgroup, VitD [OR=0.984(0.972-0.996), $P=0.01$] was protect factor for anti-HBs expression (Table 5, Figure 2).

Figure 3

The OR of age, WBC, VitD means each additional unit of these variables increases the probability of anti-HBs negative for $(OR-1) \times 100\%$. Then, variables with $P < 0.1$ in univariable logistics regression analysis were included into multivariable logistics regression. Use stepwise backward maximum likelihood method to eliminate meaningless variables ($\alpha_{in}=0.05$, $\alpha_{out}=0.10$). Except that WBC was excluded, the results were similar to those of univariate logistic (Table 6, Figure 3).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table3.xlsx](#)
- [Table4.xlsx](#)
- [Table5.xlsx](#)