



## Evaluation of Immune Response to Hepatitis B Vaccine among Malnourished Children in Yemen

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### ABSTRACT

**Objectives:** To determine the coverage rate of hepatitis B virus (HBV) vaccine and to evaluate the immune response to HBV vaccine by measuring hepatitis B surface antibody (anti-HBs) among malnourished under-five-year old children.

**Methods:** A cross-sectional study was conducted in two tertiary hospitals in Yemen; Al-Sabeen Maternity and Child Hospital in Sana'a and the Yemeni-Swedish Hospital in Taiz city in the period from March 2014 to Dec. 2014. The target population was malnourished children aged from 6 to 59 months old with a history of three HBV vaccine doses in infancy. According to the World Health Organization's definition of malnutrition, 121 malnourished children were enrolled in the study. Data of malnourished children were collected using a pre-designed, pre-tested questionnaire. Two milliliters of venous blood were taken, and anti-HBs was then tested by enzyme linked immunosorbent assay. An anti-HBs level of at least 10 IU/L was considered a successful response to the vaccine.

**Results:** The coverage rate of HBV vaccine among malnourished children was 89.3%, being higher among girls (52.1%) than boys (37.2%). Response to HBV vaccine ( $\geq 10$  IU/L) was observed in 72.2% (78/108) of children while 27.8% (30/108) of children failed to respond to the vaccine, with a statistically significant difference ( $p < 0.001$ ).

**Conclusions:** A good HBV vaccine coverage rate was found among malnourished Yemeni children, with a moderate rate of protection. Therefore, re-vaccination or administration of booster doses to a substantial proportion of vaccinated children should be considered.

**Keywords:** Hepatitis B virus, Hepatitis B vaccine, Malnutrition, Immune response, Yemen

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## 1. Introduction

Hepatitis B virus (HBV) infection poses a major health problem worldwide, with about a third of the world's population showing seropositivity as a result of current or previous infections. Approximately 400 million people are chronically infected with the virus worldwide, leading to about one million annual deaths from liver cirrhosis and hepatocellular carcinoma (1). Several previous surveys in Yemen showed high prevalence rates of hepatitis B surface antigen (HBsAg) ranging from 8% to 50% (2, 3). HBsAg prevalence rates were reported to be 26.3%, 10.5%, 5.6% and 4.8% in Socotra, Sana'a, Hajjah and Aden, respectively during the period from 2000 to 2005 (4). Chronic HBV infection develops among 90% of newborns, 29–40% of children (1–5 years old) and 5–10% of adults (5).

Primary prevention by vaccination is the most effective way for controlling HBV spread (6). Given that HBV vaccine has been available since 1982, the incidence of HBV infection and its associated morbidity and mortality declined in Taiwan and the United States (7, 8). In 1998, the World Health Organization (WHO) recommended the inclusion of HBV vaccine in the National Immunization Programme of Yemen, particularly among neonates, where vertical transmission is common, irrespective of the HBsAg prevalence (9). Injection of three doses of HBV vaccine provides a safe level of protection in 95% of infants and healthy children. However, the primary immune response to HBV vaccine decreases with increasing age, especially after 40 years old, where it may decline to 90% (10). Studies in Yemen have demonstrated the immunogenicity of HBV vaccine among healthy infants and children, where between 54.8% (9) and 83.5% (11) showed a good anti-HBs response to the pentavalent or single vaccine of  $\geq 10$  IU/L. Several factors are associated with decreased immune response to the vac-

cine, including increasing age, obesity, sex as well as genetic factors. Nutritional status is a major factor influencing both the immune response and immunodeficiency (12, 13). In developing countries, about a third of children suffer from malnutrition (15), and malnutrition has been reported as one of the most significant causes of mortality, especially among under five-year-old children (14). Malnutrition is accountable for more than 50% of the 10.8 million deaths per year among under five-year-old children and causes a death per second associated with infectious diseases in developing countries (16). Yemen has one of the highest under-five mortality rates in the world, with 77 deaths per 1,000 live births. Fifty four percent of child mortality is associated with underweight. Additionally, it has the second highest chronic malnutrition rate among children after Afghanistan (17).

Causes of malnutrition in Yemen include improper complementary food preparation, low economic status, lack of nutritional knowledge, illiteracy, lack of clean water supplies, early or delay in weaning and dependence on imported grains as the main staple (18). Malnutrition and food insecurity are further aggravated by conflicts in the country (18). Malnutrition may affect a variety of immune cells that may therefore be indicative of the nutritional status (19).

Defects in cellular and humoral immunity as well as phagocyte function disorders can be caused by protein energy malnutrition (PEM) (20, 21). In addition, reductions in the levels of the complement (except C4), secretory immunoglobulin A and cytokines can result (20, 21). Malnutrition, secondary to deficiencies in proteins, metal elements or vitamins, can lead to changes and severe atrophy in the thymus gland because of apoptosis-induced thymocyte depletion. This can lead to a persistent lympho-



cyte reduction, particularly in the immature CD4<sup>+</sup> CD8<sup>+</sup> cells, and to a decrease in cell proliferation. It is noteworthy that these conditions can be reversed following proper diet rehabilitation (22). PEM also reduces the concentrations of IgA, IgM and IgG and the production of cytokines (23). PEM-associated micronutrient deficiencies also have adverse effects on the immune response. Deficiency in zinc, iron, copper, vitamins plays an important role in reduced immune response as a result of malnutrition (24). Zinc, copper and selenium are needed to maintain and reinforce immune and antioxidant systems (25). Vitamin A stimulates cell differentiation and cytokine secretion by macrophages, including tumor necrosis factor, interleukin (IL)-1, IL-6, and IL-12 (26).

The present study aimed to determine the coverage rate of HBV vaccine among malnourished Yemeni children and to assess the immune response of those vaccinated with three doses of HBV vaccine by measuring the circulating anti-HBs levels.

## 2. Methods

### 2.1. Study population

A cross-sectional study was conducted in Al-Sabeen Maternity and Child Hospital in Sana'a and in the Yemeni-Swedish Hospital in Taiz, Yemen, in the period from March 2014 to Dec. 2014. The study was approved by the Department of Immunology, Tropical Medicine Program in Sudan Academy of Sciences, Sudan. Informed consent was obtained from the parents or guardians of children following explanation of the study goals.

The enrolled children were evaluated for the type and degree of malnutrition by anthropometric measurements, including body weight, height, and mid-upper-arm circumference (MUAC) in relation to age using regularly

validated, standard equipment. The weights were measured by scales to the nearest 10 grams and the length/height to the nearest centimeter. Three indicators were used to assess the nutritional status of children: weight-for-length, length-for-age and weight-for-age scales are  $\leq -2$  standard deviation (SD) child is at moderate and  $\leq -3$  SD is at severe malnutrition (27). Based on the WHO classification of malnutrition (27), 121 malnourished children were enrolled in the study and classified into two groups: moderate and severe malnutrition. Children's data were collected in a pre-designed, pre-tested questionnaire, including vaccination dates according to the last dose of HBV vaccine, sex and age at the time of the study.

### 2.2. Measurement of HBV markers

Blood samples were collected from selected malnourished children, aged from 6 to 59 months old, having taken the three doses of the pentavalent HBV vaccine at 6, 10 and 14 weeks of age, according to the WHO recommendations. Two milliliters of venous blood were collected, and sera were separated and frozen at  $-20$  °C until testing. All samples were investigated for anti-HBs using a fully-automated enzyme linked immunosorbent assay (ELISA) (AxSYM, Abbott, Germany). Anti-HBs antibodies were detected by sandwich ELISA using recombinant HBsAg according to the manufacturer's instructions.

HBsAg and hepatitis B core antibody (anti-HBc) were measured to differentiate current from previous infections, where all anti-HBc-positive individuals were excluded. Anti-HBs antibodies were expressed in international units per liter (IU/L). The protective immune response was defined as anti-HBs antibody levels of more than 10 IU/L (28, 29). The results were classified into four groups as follows: no response if anti-HBs level was  $<10$  IU/L, low



response if anti-HBs level was 10–100 IU/L, good response if anti-HBs level was 101–1000 IU/L and high response if anti-HBs level was >1000 IU/L.

### 2.3. Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL, USA). For qualitative data, chi-square test was used. Differences were considered statistically significant at *p* values <0.05.

## 3. Results

A total of 121 malnourished children with a mean age of 23.11 ± 16.8 months were included in this study; 42.1% (51/121) were boys and 57.9% (70/121) were girls. Vaccinated children represented 89.3% (108/121) of malnourished children, and the HBV vaccine coverage rate was higher (52.1%) among girls than boys (37.2%) (Table 1). In addition, 72.2% (78/108) of vaccinated children were responders (anti-HBs level ≥10 IU/L) while 27.8% (30/108) were non-responders (anti-HBs levels <10 IU/L). The average protective rate in all age group was 72.2%, with a significantly higher rate (*p* = 0.001) among younger age groups (Table 2). The age group of less than one year showed significantly higher anti-HBs levels than the age group of 4-5 years (*p* <0.001) (Table 3). Of the vaccinated children, 41.7% (45/108) had moderate malnutrition and 58.3% (68/108) had severe malnutrition. Sero-protection was found among 72.2% (78/108) of vaccinated children. Protective rates in children with moderate and severe malnutrition were 24.1% and 48.1%, respectively, with a statistically significant difference (*p* = 0.043) (Table 4).

**Table 1.** The HBV vaccine coverage rate among malnourished children according to sex

Status	Boys (n=51)	Girls (n=70)	Total (n=121)	<i>p</i> value*
	No.(%)	No. (%)	No. (%)	
Vaccinated	45 (37.2)	63 (52.1)	108 (89.3)	0.700
Non-vaccinated	6 (5.0)	7 (5.8)	13 (10.7)	
<b>Total</b>	<b>51 (42.1)</b>	<b>70 (57.9)</b>	<b>121 (100)</b>	

\* Calculated for chi-square

**Table 2.** Comparison of anti-HBs antibody levels among malnourished children according to age

Age group (years)	Anti-HBs <10 IU/L	Anti-HBs ≥10 IU/L	Total	<i>p</i> value*
	No. (%)	No. (%)		
<1	5 (13.9)	31 (86.1)	36	0.023
1-2	11 (25.0)	33 (75.0)	44	0.593
>2-3	2 (25.0)	6 (75.0)	8	0.850
>3-4	2 (28.6)	5 (71.4)	7	0.960
4-5	10 (76.9)	3 (23.1)	13	<0.001
<b>Total</b>	<b>30 (27.8)</b>	<b>78 (72.2)</b>	<b>108</b>	<b>&lt;0.001</b>

\* Calculated for chi-square

**Table 3.** Comparison of anti-HBs antibody levels between less than one-year-old and more than one-year-old malnourished children in Sana'a (2014)

Age group (years)	Anti-HBs <10 IU/L	Anti-HBs ≥10 IU/L	Total	<i>p</i> value*
	No. (%)	No. (%)		
<1	5 (13.9)	31 (86.1)	36	<0.001
4-5	10 (76.9)	3 (23.1)	13	
<b>Total</b>	<b>15 (30.6)</b>	<b>78 (72.2)</b>	<b>49</b>	

\* Calculated for chi-square

**Table 4.** Anti-HBs levels among vaccinated malnourished children in Sana'a (2014) according to malnutrition severity

Anti-HBs level (IU/L)	Malnutrition severity		Total No. (%)	<i>p</i> value*
	Severe acute malnutrition No.(%)	Moderate acute malnutrition No.(%)		
<10	11 (10.2)	19 (17.6)	30(27.8)	0.043
10-100	21 (19.4)	11 (10.2)	32 (29.6)	
101-1000	19 (17.6)	10 (9.3)	29 (26.9)	
>1000	12 (11.1)	5 (4.6)	17 (15.7)	
<b>Total</b>	<b>63(58.3)</b>	<b>45 (41.7)</b>	<b>108(100.0)</b>	

\* Calculated for chi-square



#### 4. Discussion

In the present study, the HBV vaccination coverage rate among malnourished Yemeni children was 89.3%. This finding is higher than that (69.9%) of a previous study conducted by Al-Shamahy et al. (9) on healthy children in Sana'a. This difference may possibly be due to the difference in the times at which the two studies were conducted and to the enhanced response to the Ministry of Public Health and Population calls (educational programs and vaccination campaigns). In general, the health status has greatly improved in Yemen over the years due to better socio-economic levels, educational status and health awareness. However, the finding of the present study is lower than those reported from other HBV endemic countries, where HBV vaccine coverage rates among children ranged from 90–98% (30, 31).

In this study, there was a significant difference in protection rate at the various annual intervals (1–5 years) since vaccination in two studies; annual titer reduction in children under seven years old was 10.2% (13). Zhou et al. (32) reported that protective levels of antibodies decrease with time. Furthermore, a recent study showed that in healthy Yemeni people, the levels of anti-HBs antibodies decreased with time (9). Increasing age was correlated with a decreasing level of protection rate. The response rate of anti-HBs declined from 86.1% in the < one-year age group to 23.1% in > 4–5-year age groups. This result is similar to those of other studies conducted in Yemen (9), Saudi Arabia (33), China (34) and Europe (35). Immunosenescence as a result of continuous immune response deterioration results in a decreased response to vaccines. Decrease in *de novo* production of B and T cells is a well-described alteration related to increasing age. In addition, the memory cell accumulation and loss of diversity in antigen specificities due to a

lifetime of exposure to pathogens have also been described (36).

The mean anti-HBs level in the present study was 304.54 IU/L, and the rate of seroprotection was 72.2%. Such rate is lower than that reported by Sallam et al. (11) in a study conducted in Sana'a, where 83.5% of healthy children were with anti-HBs seroprotective levels (11). However, the seroprotective rate in the present study is higher than that (54.8%) among healthy children in Sana'a (9). Variable seroprotective rates have been reported from different countries; 96.7% in Turkey (37), 86% in Taiwan (38), 57.2% in Egypt (39) and 41% in the United States (40).

In our study, nutritional status was found to affect anti-HBs levels, with a significant association between anti-HBs levels and the severity of malnutrition. This is similar to other studies. For instance, Rey et al. (41) found that nutritional status was significantly associated with response to HBV vaccination in Senegal and Cameroon, where 85% of children with normal nutritional status were protected (anti-HBs  $\geq 10$  IU/L) versus 60% with moderate to severe malnutrition. In Iran, Karimi et al. (42) found a significant decrease in the immune response to HBV vaccine in malnourished children (60.2%) and that severe acute malnutrition produced a higher protective immune response than moderate acute malnutrition. Losonsky et al. (43) concluded that low and poor weight gains in the first 6 months of life were associated with reduced immunogenicity (71%) following three doses of HBV vaccine in the United States. Wang (44) reported that the response to HBV vaccine in low socio-economic areas was low in Taiwan. Experimentally, rats with PEM were found not to respond to HBV vaccination in comparison to control rats. In a histopathology study, PEM was found to cause a decrease in dendritic cells and their stimulatory functions on T cells (45). Aria et al. (46) showed a negative effect of mal-



nutrition, immune deficiency and drug addiction on HBV vaccine immune response. In a similar fashion, antibody responses to H. influenzae type b conjugate vaccine was found to be lower in Kenyan children with stunting and chronic malnutrition than those in well-nourished infants (47). These contradictory findings on survival of vaccine can be due to ethnic differences or non-apparent exposure to HBV in endemic areas.

A descriptive study conducted by Charareewong et al. (48) on 25 premature infants showed the seroconversion rate was high 88%. El-Jamal et al. (49) reported a 100% protection rate among Egyptian healthy infants two months after the last HBV vaccine dose compared to 87% among protein energy malnourished ones; however, the difference was not significant. However, Muhammad et al. (50) concluded that the nutritional status had no effect on the immune response of infants to HBV vaccine. These differences stress the necessity for further studies with larger sample sizes and consideration of possible factors such as age, sex, race, site of injection, nutritional status and vaccine brand. Obesity is a type of malnutrition, and some studies showed that obesity was a predicting factor for a weak response to HBV vaccine (51, 52). Frequent studies investigated the effect of malnutrition on response to HBV vaccine in hemodialysis patients. The impact of malnutrition on seroconversion has been shown (51). We postulate that malnutrition in under five-year-old children cannot prevent a competent immune response to HBV vaccine but may reduce such response. Similar to our findings were reported in a study conducted in Guatemala (53).

## 5. Conclusions

A good HBV vaccine coverage rate was found among malnourished Yemeni children, with a moderate rate of protection. This indi-

cates that nutritional status affects the immune response to HBV vaccine and seroprotection rate. Although these results are for malnutrition as a whole, our small sample size makes it impossible to conclude definitively on the severity of malnutrition and impact on the immune response. Therefore, further research with a larger sample size and case-control studies are required. Re-vaccination or administration of booster doses to a substantial proportion of vaccinated children in Yemen should be considered.

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## Competing interests

The author declares that he has no competing interests associated with this article.

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