Low Immunogenicity of Recombinant Hepatitis B Vaccine Derived from *Hansenula polymorpha* in Adults Aged Over 40 Years

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Abstract. The Brazilian recombinant hepatitis B vaccine (VrHB-IB) is based on the expression of the recombinant antigen in *Hansenula polymorpha* yeast cells. Currently, data on the immunogenicity of this vaccine in older adults are nonexistent. This study aimed to evaluate the immunogenicity of VrHB-IB in adults over 40 years of age. From May to October 2011, 235 rural settlers between 2 and 93 years of age from the State of Goiás in Brazil were eligible for vaccination. Of these, 180 accepted the first dose of the vaccine and 106 (58.9%) completed the vaccination schedule. Multivariate analysis revealed that individuals ≥ 40 years of age responded significantly less well to vaccination than younger adults. Also, a greater proportion of male nonresponders was observed (versus women; $P = 0.02$). These results point to the need for better evaluation of the immunogenicity of VrHB-IB in older adults.

Vaccination against hepatitis B is the safest and most effective method of reducing the incidence and prevalence of infection with hepatitis B virus (HBV).¹ Indeed, HBV vaccination confers protection in over 90% of subjects who receive it.² The first-generation HBV vaccine, which was produced from human HBV surface antigen (HBsAg) plasma from chronically infected patients, became commercially available in the early 1980s and is still used in some countries.³ The second-generation vaccine, also marketed in the 1980s, is produced using recombinant DNA technology. This vaccine is safe and effective, and allows for unlimited production and widespread distribution throughout the world. Currently, many countries produce the HBV vaccine using this technology, and various expression systems have been used to obtain recombinant HBsAg.^{1,4} Engerix-B, the gold-standard vaccine, uses *Saccharomyces cerevisiae* as the expression system for recombinant HBsAg.¹

In Brazil, the Brazilian recombinant hepatitis B vaccine (VrHB-IB) is obtained by culturing genetically engineered *Hansenula polymorpha* yeast cells with the HBsAg gene. Initially, this vaccine was produced as a recombinant antigen (concentration 20 $\mu\text{g/mL}$). However, research has shown that in adults 30–40 years of age, the immunogenicity of the Brazilian vaccine is not equivalent to the gold-standard vaccine (Engerix B),⁵ and the concentration of HBsAg was therefore increased to 25 $\mu\text{g/mL}$. A clinical trial comparing the immunogenicity of VrHB-B with Engerix-B in patients in this age group found that the vaccine produced in Brazil at the current concentration is equivalent to the gold standard.⁶

The aim of our study was to evaluate the immunogenicity of VrHB-IB vaccination in rural settlers in central Brazil. Rural settlers who were susceptible to HBV comprised the vaccination group, and most of them were > 40 years of age. At present, published studies on the immunogenicity of the Brazilian HBV vaccine in adults in this age group are rare.

From May to October 2011, 467 rural settlers from the southwest region of the State of Goiás, central Brazil, were

recruited. All agreed to participate in the study and signed a consent form. The participants were tested initially for HBV markers (HBsAg, anti-HBc, and anti-HBs). Those who were negative for all serologic markers and had not reported a previous vaccination history for hepatitis B were identified as susceptible to the virus and eligible for vaccination. This study was approved by the ethics committee of the Federal University of Goiás (protocol CEPMHA/HC/UFG No. 127/2010).

The following Brazilian VrHB-IB vaccine lot numbers (Instituto Butantan, São Paulo, Brazil) were used: 1006117 and 1006116. Each mL of vaccine contains 25 $\mu\text{g/mL}$ of recombinant HBsAg expressed in *H. polymorpha*. The vaccine was administered intramuscularly in three doses, with an interval of 1 month between the first and second doses, and 5 months between the second and third doses. Forty-five to 60 days after the third vaccination dose, eligible individuals were contacted for blood collection and quantitative detection of anti-HBs markers, which were measured using a microparticle enzyme immunoassay (AxSYM® Ausab®; Abbott, Wiesbaden, Germany). Seropositivity was defined as an anti-HBs antibody concentration ≥ 1 mIU/mL, and seroprotection as ≥ 10 mIU/mL.

Data were analyzed using the statistical program, STATA 11 (StataCorp., College Station, TX). Geometric mean anti-HBs titers were calculated using a confidence interval (CI) of 95%. The χ^2 test and Fisher's exact test were used to test the significance of the differences between the proportions. P values of < 0.05 were considered statistically significant. A total of 77 subjects were sufficient to achieve 80% power to detect a difference in seroprotection rate of 35% between nonresponder and responder groups. The significance level of the test was 0.05.

Of the total study participants (467), 51 (10.9%; 95% CI = 8.4–14.1) presented some serological markers of exposure to infection by HBV. In 90 individuals (19.3%; 95% CI = 15.9–23.1), positivity was observed for isolated anti-HBs markers, indicating previous vaccination against hepatitis B.

Ninety-one individuals reported previous hepatitis B vaccination histories. Therefore, nearly half of the participants (235/467) between 2 and 93 years of age were susceptible to infection and were eligible for vaccination. Of those, 180 accepted the first dose of the vaccine and 106 (58.9%;

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FIGURE 1. Flow chart for hepatitis B vaccination performed in rural settlers in the southwest region of central Brazil, 2011.

106/180) completed the vaccination schedule. The vaccine response was evaluated for 77 individuals (Figure 1).

The characteristics of the individuals who completed the vaccination series and were evaluated for their vaccine responses were similar to those susceptible to hepatitis B, except for age and marital status (Table 1). Over half of the individuals were female (51.9%) and almost half had up to 4 years of primary-level schooling (49.3%). Also, most of these were married (87%), native to the midwest region of Brazil (71.4%), and > 40 years of age (71.4%). Only 68.8% (53/77) had protective anti-HBs titers, and of these, 30 developed titers > 100 mIU/mL. The geometric mean of the anti-HBs antibodies was 27.92 mIU/mL. Multivariate analysis revealed that individuals ≥ 40 years of age responded significantly less well to vaccination than young adults. Also, men comprised the greater proportion of nonresponders (versus women; $P = 0.02$) (Table 2).

In 1998, the Ministry of Health of Brazil began offering the HBV vaccine free of charge for babies up to 1 year of age, a service that was gradually extended to older people. Nowadays, HBV vaccine is offered to the entire population, regardless of age or vulnerability.⁷ Despite this policy, differences exist in the vaccination uptake across different age groups and low immunization rates are apparent in some groups.^{8,9} Also, in older age groups, the immunogenicity of this vaccine is not known, and there are many

factors that may affect how immunogenic the vaccine is in these groups.¹

As has been observed for the gold-standard vaccine by other authors,^{10–12} sex represents a statistically significant difference in terms of vaccine responders and nonresponders. Different biological characteristics and behaviors contribute to men developing a weaker immune response to vaccination than women.¹³

The concentration of VrHB-IB is greater than recombinant HBsAg to the gold standard, Engerix-B.^{6,14,15} According to Moraes and others,⁶ with an HBsAg concentration of 25 µg/mL, the immunogenicity of the vaccine produced in Brazil for adults 31–40 years of age is equivalent to the gold standard, for which 98.6% of the vaccinees developed protective titers. In fact, in subjects < 40 years of age, the vaccine response was equivalent to that reported by other authors using vaccines expressed in *S. cerevisiae* (Engerix-B)¹⁶ and *H. polymorpha* (GeneVac B).¹⁵ In older individuals, surprisingly, there was a much lower percentage of seroprotection than desired in an age range of the Brazilian population that does not present comparative data. Among those aged 40–49 and 50–59 years, only 61.9% and 55.9% were responders, respectively. Investigations using the gold-standard vaccine illustrate higher response rates in older adults,^{10,14} as was also found in a study using the GeneVac-B vaccine.¹⁵

TABLE 1
Characteristics of participants eligible for HBV vaccination (*N* = 235), central Brazil, 2011

Variables	Participants who refused vaccination (%)	Participants who started the vaccine series (%)	Participants vaccinated and evaluated for immunogenicity (%)	Total (%)	<i>P</i> value
	<i>N</i> = 55	<i>N</i> = 103	<i>N</i> = 77		
Gender					
Female	22 (40)	52 (50.5)	40 (51.9)	114 (48.5)	0.346
Male	33 (60)	51 (49.5)	37 (48.1)	121 (51.5)	
Age (years; range 2–93)					
≤ 39	41 (74.5)	33 (32)	22 (28.6)	96 (40.9)	0.000
40–49	7 (12.7)	23 (22.3)	21 (27.3)	51 (21.7)	
≥ 50	7 (12.7)	47 (45.6)	34 (44.1)	88 (37.4)	
Civil status					
Married/steady partner	15 (27.3)	81 (78.6)	67 (87)	163 (69.4)	0.000
Single	40 (72.7)	16 (15.5)	8 (10.4)	64 (27.2)	
Separated	0 (0)	2 (1.9)	1 (1.3)	3 (1.3)	
Widowed	0 (0)	4 (3.9)	1 (1.3)	5 (2.1)	
Education (years)					
≤ 4	19 (40.4)	48 (46.6)	38 (49.3)	105 (46.3)	0.718
5–8	21 (44.7)	40 (38.8)	25 (32.5)	86 (37.9)	
≥ 9	7 (14.9)	15 (14.6)	14 (18.2)	36 (15.9)	
NA: 8					
Region					
Midwestern	47 (85.5)	84 (81.6)	55 (71.4)	186 (79.1)	0.380
Northeast	4 (7.3)	7 (6.8)	14 (18.2)	25 (10.6)	
North	0 (0)	0 (0)	1 (1.3)	1 (0.4)	
Southeast	1 (6.7)	9 (8.7)	5 (6.5)	15 (6.4)	
South	3 (5.5)	3 (2.9)	2 (2.6)	8 (3.4)	
Tobacco user					
No	28 (87.5)	74 (72.5)	54 (70.1)	156 (73.9)	0.154
Yes	4 (12.5)	28 (27.5)	23 (29.9)	55 (26.1)	
NA: 24					

HBV = hepatitis B virus; NA = not applicable.

Indeed, in a multicenter study of 144 individuals ≥ 50 years of age who received three doses of Engerix-B, 84% developed protective titers.¹⁴ In the Netherlands, 11,439 individuals were evaluated after completing the vaccination schedule and, for men and women, a response rate of 90% was found for ages 41 and 53, respectively. In the same study, this rate declined to 80% for male and female vaccinees 54 and 68 years of age, respectively.¹⁰ Concerning the GeneVac-B vaccine, a greater response rate was also found in older individuals compared with the data from this investigation. Those aged 40–49 years and 50–57 years, which represents 93% and 85% of the participants, respectively, developed protective titers.¹⁵

Immune system aging, which is referred to as immunosenescence, affects responses to the HBV vaccine¹⁷; hence, the immunogenicity rate for this vaccine in older individuals is generally lower. However, our findings suggest that the immunogenicity of the Brazilian recombinant vaccine is even

lower in individuals > 40 years of age compared with the gold-standard vaccine and others.^{6,18} The greatest discrepancy in protection rates was found between the two vaccines that are expressed in *H. polymorpha*, and which also use the same adsorbent (aluminum hydroxide).^{6,18} Therefore, it seems likely that factors such as storage, transport, and packaging may influence the effectiveness of the Brazilian vaccine. However, all these factors were thoroughly checked during the developmental stages of this investigation, according to Brazilian recommendations.¹⁹

This study has some limitations. Rural settlers are not representative of the national population as a whole. Also, budget constraints meant we were unable to form a control group and evaluate the response rates using the gold-standard vaccine; however, our findings for subjects < 40 years of age were similar to those of the gold-standard vaccine.¹⁶ Factors that may have interfered with vaccine responses, such as obesity and immunosuppression, were not investigated.

TABLE 2
Characteristics associated with seroprotection after vaccination schedule for hepatitis B in 77 individuals, central Brazil, 2011

	Univariate analysis			Multivariate analysis*	
	Responder	Nonresponder	P value		
	N = 53 (%)	N = 24 (%)		OR (95% CI)	P value
Age (years)			0.003		
≤ 39	21 (39.6)	1 (4.2)		1.0	
40–49	13 (24.5)	8 (33.3)		11.4 (1.1–115.6)	0.04
≥ 50	19 (35.9)	15 (62.5)		14.2 (1.6–124.9)	0.02
Tobacco user	11 (20.8)	12 (50.0)	0.009	1.55 (0.8–8.5)	0.12
Male	20 (37.7)	17 (70.8)	0.007	3.8 (1.2–12.3)	0.02

CI = confidence interval; OR = odds ratio.

*Logistic regression.

Furthermore, the vaccine response was evaluated in only 77 individuals who represent 43% of those who received the first vaccine dose. Nevertheless, these results represent the first data on Brazilian vaccine immune responses among people ≥ 40 years of age, and point to the need for better evaluation of the immunogenicity of the Brazilian HBV vaccine in older adults. With the gradual proliferation of such a vaccine, the burden of infection is moving to older age groups, whereas the results of this study suggest that poor vaccine responses occur among individuals in these groups.

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