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## HEPATITIS B VACCINE IN INFANTS: A RANDOMIZED CONTROLLED TRIAL COMPARING GLUTEAL VERSUS ANTEROLATERAL THIGH MUSCLE ADMINISTRATION

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### SUMMARY

A significantly diminished antibody response to hepatitis B vaccine has been demonstrated in adults when the buttock is used as the injection site. However, in Brazil, the buttock continues to be recommended as site of injection for intramuscular administration of vaccines in infants. In this age group, there are no controlled studies evaluating the immunogenicity of the hepatitis B vaccine when administered at this site. In the present study, 258 infants were randomized to receive the hepatitis B vaccine either in the buttock (n = 123) or in the anterolateral thigh muscle (n = 135). The immunization schedule consisted of three doses of hepatitis B vaccine (Engerix B<sup>®</sup>, 10 µg) at 2, 4 and 9 months of age. There were no significant differences in the proportion of seroconversion (99.3% x 99.2%), or in the geometric mean titer of ELISA anti-HBs (1,862.1 x 1,229.0 mIU/mL) between the two groups. This study demonstrates that a satisfactory serological response can be obtained when the hepatitis B vaccine is administered intramuscularly into the buttock.

**KEYWORDS:** Hepatitis B; Vaccine efficacy; Injection; Intramuscular; Gluteal injection; Infant.

### INTRODUCTION

Early studies on the response to hepatitis B vaccine have demonstrated a 96% seroconversion rate in the target population<sup>28,29</sup>. However, in 1984 reports appeared of a serological efficacy of only 63 to 68% among vaccinated health workers<sup>22,26</sup>. In 1985, a surveillance study by the Centers for Disease Control and Prevention (CDC) in 57 hospitals in the United States showed a significantly higher antibody response against the surface antigen of the hepatitis B virus (anti-HBs) in hospitals where the deltoid muscle was used as the injection site compared to the buttocks, 93% and 81% respectively<sup>3</sup>. Based on these findings, administration of hepatitis B vaccine in the buttock is no longer recommended at any age. Administration into the deltoid muscle is recommended for older children and adults, while the anterolateral thigh muscle is preferred for infants and toddlers. In 1998, the hepatitis B vaccine was introduced in the National Immunization Program of the Ministry of Health of Brazil for children under one year of age in all country and for children under 15 years of age in the States of Santa Catarina, Paraná, Distrito Federal and in all the Amazonian region<sup>10</sup>. For infants the anterolateral thigh muscle has been recommended as the injection site. However, in Brazil, the application of intramuscular thigh injections still is not a well-established routine since the buttock continues to be recommended as site of injection for DPT vaccine<sup>11</sup>. The most recent data available from the Ministry of Health of Brazil show that more than 14 million doses of DPT were administered to children under 18 months of age in 1998<sup>27</sup>. A concern has been raised whether turning the anterolateral thigh muscle into the mandatory injection site for hepatitis B administration could result in

additional difficulties in implementing children's vaccination programs due to cultural and technical problems.

All studies reporting reduced serological efficacy of hepatitis B vaccine administered in the buttocks have been conducted on adults<sup>1,3,13,18,19,23,31,34</sup>. In this age group, an inversely proportional correlation between a high weight and height index and a satisfactory anti-HBs response has been noted<sup>35</sup>. The present study was carried out to evaluate the serological efficacy of hepatitis B vaccination in infants by comparing quantitative anti-HBs levels as a consequence of the injection site used, taking into consideration age, sex and nutritional status.

### METHODS

**Study design:** A randomized controlled trial.

**Calculation of sample size:** The sample size for the two groups was calculated on the basis of a CDC study on adults<sup>3</sup> that demonstrated a serologic efficacy of 93.9% in vaccinated subjects who received the hepatitis B vaccine in the deltoid, and an efficacy of 81.0% in subjects receiving the vaccine in the buttock. Considering a 5% type I error and 90% statistical power, 157 children were recommended for each group.

**Randomization:** A total of 314 sealed envelopes containing 156 filling cards for group A (buttock) and 158 filling cards for group B (anterolateral thigh muscle) were mixed by shuffling and then numbering at random from 1 to 314. After the infants' guardians gave written,

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informed consent, the envelopes were sequentially opened according to admission to the study. Information was then obtained regarding the group to which the infants had been assigned.

**Study population:** The participants were selected from infants aged two to four months whose parents spontaneously sought assistance at one of the basic health units in the City of São Paulo where routine medical visits are performed and the vaccines of the official immunization program are provided. The study was conducted from January 1997 to October 1998. The hepatitis B vaccine was not available to the public health network when the study was performed. Infants whose parents would not be available for follow-up or who did not agree with the randomization of the injection site were not included in the study. Children with a previous vaccination against hepatitis B, a history of blood or immunoglobulin transfusion or of prolonged treatment with corticosteroids, the presence of a clinical situation that would contraindicate routine immunization, or the children of mothers known or suspected to carry HIV or hepatitis B virus infection were also excluded. The parents signed an informed consent form and the study was approved by the Ethical Research Committee of the University of São Paulo School of Medicine.

**Intervention plan:** The Engerix B<sup>®</sup> vaccine (Smith Kline Beecham, Rixensart, Belgium) as a 10 µg dose was injected IM into the upper, outer right quadrant of the buttock (Group A) or into the anterolateral surface of the right thigh (Group B). The nurses in charge of vaccine administration were trained to apply IM injections into the anterolateral thigh muscle. Needles measuring 30 or 25 mm in length by 0.7 mm in diameter were used for the buttock and anterolateral thigh muscle injections, respectively. The vaccine was stored at 2-8 °C.

**Immunization schedule:** The immunization schedule consisted of three applications of hepatitis B vaccine (Engerix B<sup>®</sup>, 10 µg) at 2, 4 and 9 months of age. As the hepatitis B vaccine was not provided by the National Immunization Program when the study was carried out, this immunization schedule was established to limit possible interference with other routine vaccines. Concomitantly with the first and second applications of hepatitis B vaccine, the infants also received the oral polio vaccine (OPV) and diphtheria/tetanus/pertussis (DTP) vaccines, the latter being injected into the upper, outer left quadrant of the buttock. The measles vaccine was applied simultaneously with the third dose of the hepatitis B vaccine. Different syringes, needles, and injection sites were used for each vaccine. The vaccine against *Haemophilus influenzae* type-b, also injected into the anterolateral thigh muscle, was not available to the public health network of the State of São Paulo during the study. The collection of a post-vaccinal blood sample was scheduled for the 15<sup>th</sup> month of life. No blood samples were collected before vaccination since infants previously vaccinated against hepatitis B virus were excluded from the study. To detect infants eventually infected with the wild virus, we tested the serum samples from non-responding infants (anti-HBs levels < 10 mIU/mL) for the presence of the core antigen antibody (anti-HBc) of the hepatitis B virus (AxSYM<sup>®</sup> – Abbott Diagnostics, Abbott Park, IL, USA).

**Detection of anti-HBs:** An automated, quantitative immunoenzymatic assay was performed to detect anti-HBs (Cobas Core II<sup>®</sup>, Roche Diagnostic Systems, Basel, Switzerland). The plasma concentration of anti-HBs is given as mIU/mL. The anti-HBs response

was considered to be protective when it corresponded to  $\geq 10$  mIU/mL and cases with antibody levels < 10 mIU/mL were considered to be non-responders<sup>3</sup>. Samples with anti-HBs levels above 150 mIU/mL were first diluted 1:10. Samples with titers higher than 1,500 mIU/mL were further diluted to 1:100.

**Anthropometric analysis:** The nutritional status of the study population was assessed by anthropometry. The calculations were based on the standard growth curves elaborated by the U. S. National Center for Health Statistics (NCHS) and by the CDC, using data from the Field Research Institute and U. S. Health Examination Surveys recommended by the World Health Organization<sup>37</sup>, with the aid of the Epi Info-6.0 nutritional anthropometry software. From the basic data on weight, sex, age and height obtained during selection of the study population at the time of the first application of hepatitis B vaccine, we calculated the two major anthropometric indices, i.e., weight for height (WH) and height for age (HA). These indices are reported in terms of Z-scores (WHZ and HAZ)<sup>37</sup>. Thus, once the mean values for WHZ and HAZ were obtained, it became possible to compare the two groups studied. The relationship between nutritional status and antibody levels reached was also evaluated (anti-HBs < 100 and anti-HBs > 100 mIU/mL).

**Statistical analysis:** First descriptive analysis (frequency distribution, arithmetic and geometric means, medians and standard deviation [SD] ) were calculated. The Student t-test was calculated to compare means of normally distributed samples (age, weight and length); when not applicable, Wilcoxon-Mann-Whitney U test was used<sup>25</sup>. Quantitative variables were dichotomized into two categories, the cut-off point lying close to the median. The efficacy of the vaccine in each group was assessed from the proportion of non-responders according to the injection site studied. To analyze the variable anti-HBs > 10 mIU/mL according to the group studied, we also calculated the geometric mean for antibody levels, which is not affected by extreme values. The group of infants with anti-HBs levels < 100 mIU/mL was analyzed separately by univariate analysis to detect typical characteristics, and later by unconditional logistic regression to control for possible confounding variables<sup>17</sup>. The strength of the association between variables was estimated by calculating the odds ratio (OR) and 95% confidence interval (95% CI). Statistical significance was evaluated by the likelihood ratio test (LR).

## RESULTS

Of 314 infants, 258 (82.2%) completed all stages of the study. No significant differences were observed between the group that concluded the study and the infants lost to follow-up with respect to age, sex, weight, height, and vaccine application site. Of those who remained in the study (n = 258), the mean interval (SD) between the 1<sup>st</sup> and the 2<sup>nd</sup> vaccine applications was 67.0 days (15.8 days), that between the 2<sup>nd</sup> and 3<sup>rd</sup> doses was 175.8 days (40.7 days), and that between the 3<sup>rd</sup> dose and blood collection was 144.5 days (50.5 days). No significant differences were seen in the characteristics of the infants who received the vaccine in the anterolateral thigh muscle or in the buttock (Table 1).

Only 2 of the 258 infants presented non-protective (< 10 mIU/mL) post-vaccinal antibody levels. Each non-responder case belonged to one of the two groups, resulting in a serological efficacy of 99.3% (134/135) for the anterolateral thigh muscle group and an efficacy of 99.2% (122/

**Table 1**

Characteristics of the infants receiving hepatitis B vaccine in the anterolateral thigh muscle or in the buttock\* and intervals (days) between 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> application of vaccine and between 3<sup>rd</sup> vaccine application and blood post-vaccinal collection (mean ±SD)

Variable	Vaccination site	
	Anterolateral thigh muscle	Buttock
Number	135	123
Sex (female)	63 (46.7%)	63 (51.2%)
Age (days)	74.1 ± 15.2	73.6 ± 14.9
Weight (Kg)	5.5 ± 0.8	5.5 ± 0.9
Length (cm)	56.7 ± 3.1	56.8 ± 3.2
Dose interval (1 <sup>st</sup> and 2 <sup>nd</sup> )	67.9 ± 17.1	66.4 ± 14.1
Dose interval (2 <sup>nd</sup> and 3 <sup>rd</sup> )	173.4 ± 36.0	178.5 ± 45.3
Interval between 3 <sup>rd</sup> dose and blood collection	144.6 ± 51.1	144.4 ± 50.2

\* The differences are not statistically significant

123) for the buttock group. No anti-HBc was found in these cases. The geometric mean titer of anti-HBs was 1,229.0 mIU/mL for the buttock group and 1,862.1 mIU/mL for the anterolateral thigh muscle group ( $p = 0.06$ ). The median anti-HBs titer was 2,051 mIU/mL for the anterolateral thigh muscle group and 1,485 mIU/mL for the buttock group ( $p = 0.14$ ).

Twenty-two of the 258 infants (8.5%) had anti-HBs levels < 100 mIU/mL. Of these, seven belonged to the 135 infants who received the vaccine in the anterolateral thigh muscle (5.2%) and 15 belonged to the group of 123 infants who received the vaccine in the buttock (12.2%). This difference was statistically significant (OR = 2.54,  $p = 0.04$ ). Univariate analysis of the main characteristics of the infants with anti-HBs levels < 100 mIU/mL (injection site, nutritional status, interval between vaccine applications and interval between the 3<sup>rd</sup> vaccine dose and blood collection) showed that the single factor related to this type of response was the vaccination site ( $p = 0.04$ ). Multivariate analysis for the control of possible confounding variables (age, sex, weight, length, interval between vaccine applications, and interval between the 3<sup>rd</sup> vaccine dose and blood collection) showed that injection into the buttock continued to be a significant independent factor associated with anti-HBs levels < 100 mIU/mL (OR = 2.60,  $p = 0.04$ ). Eighty-one of the infants studied exhibited anti-HBs levels > 5,000 mIU/mL after vaccination, 36 belonging to the buttock group and 45 to the anterolateral thigh muscle group ( $p = 0.08$ ).

## DISCUSSION

The hepatitis B vaccine administered IM to infants was highly effective regardless of the application site. Only two infants did not exhibit seroconversion at the end of the study, each belonging to a different group. No difference in the geometric mean of anti-HBs levels > 10 mIU/mL was detected between the groups vaccinated in the anterolateral thigh muscle or in the buttock.

These results agree with previous reports on the high immunogenicity of hepatitis B vaccine in children<sup>15,16,36</sup>, but differ from reports of vaccine failure after administration in the buttocks of adults<sup>1,3,13,18,19,23,31,34</sup>. A previous study, analyzing the risk factors associated with a diminished response to hepatitis B vaccine among health professionals vaccinated in the buttocks, demonstrated that obese individuals presented an unsatisfactory immunological response<sup>35</sup>. A study using pelvic computerized tomography demonstrated that most of the IM injections in the buttocks of adults actually resulted in the inadvertent application into adipose tissue in 85.0% of males and at least 95.0% of females<sup>6</sup>. These results were extrapolated to infants and children, and recommendations were made not to inject hepatitis B vaccine into the buttocks of adults or children<sup>3</sup>.

In the present study, the WH and HA indices, expressed as Z-scores<sup>37</sup>, were similar in the two groups studied, even for infants presenting anti-HBs levels < 100 mIU/mL. Comparison of the risk of vaccine failure (anti-HBs < 10 mIU/mL) with respect to the nutritional profile of each study group was not performed due to the small number of infants presenting this response (one child in each group).

The fact that the two groups showed a similar proportion of seroconversion may be of practical importance since not all public health units in developing countries are staffed by workers trained in administering IM injections into the anterolateral thigh muscle. If the buttock is considered a possible alternative for hepatitis B vaccine administration, many operational and cultural problems may be avoided, eliminating possible delays in the implementation of infant immunization against hepatitis B.

No significant difference in anti-HBs levels > 5,000 mIU/ml was detected between groups. However, when the number of infants with anti-HBs levels < 100 mIU/mL was analyzed, a significant association was obtained between this parameter and the vaccine administration site ( $p = 0.04$ ). In the multivariate analysis, the only variable associated with anti-HBs levels < 100 mIU/mL was the vaccine application site (OR = 2.54, 95% CI = 1.02-6.76). However, the prevalence of infants with post-vaccination levels < 100 mIU/mL was low, both in the study population as a whole (8.5%) and in each group specifically (5.2% for the group vaccinated in the anterolateral thigh muscle and 12.2% for the group vaccinated in the buttock).

The clinical significance of this response pattern to hepatitis B vaccine is not well defined. Some investigators believe that anti-HBs levels after vaccination should exceed 100 mIU/mL since there are reports of individuals with anti-HBs levels < 100mIU/mL who present clinical or subclinical hepatitis B virus infection with transiently positive antigenemia and hepatitis B virus DNA<sup>2,5</sup>. Other investigators prefer the use of terms such as "seroconverted" for subjects who exhibit an anti-HBs response > 10 mIU/mL, as opposed to the term "seroprotected", which is reserved for those whose anti-HBs levels exceed 100 mIU/mL<sup>4,5,14</sup>.

Studies involving the clinical and laboratory monitoring of populations at a high risk of acquiring the hepatitis B virus have demonstrated that the hepatitis B vaccine induces a long-lasting protective immune response. Natural boosters occur frequently and, due to an anamnestic type response, even individuals considered to be low

responders (anti-HBs < 100 mIU/mL) continue to be protected against chronic hepatitis B virus infection for years<sup>8,21,24, 32</sup>.

Additional booster doses of hepatitis B vaccine are not recommended by the World Health Organization. Recent studies have shown that vaccine-dependent immunity is of long duration, suggesting that there is no need to administer booster doses of the vaccine to prevent a fall in or disappearance of anti-HBs levels over the years<sup>21,33</sup>.

The administration of IM injections into the buttock is believed to involve greater risk than that into the anterolateral thigh muscle because of possible damage to the sciatic nerve. However, in most reported cases of sciatic nerve palsies in infants and children after gluteal injections there was evidence of direct injection of neurotoxic material, such as antibiotics, bismuth and quinine, into or near the nerve<sup>7,12</sup>. Otherwise, there are also anecdotal reports of complications following intramuscular injections into the anterolateral thigh muscle, including muscle necrosis deep to the fascia lata, acute chemical synovitis, and fibrosis and contracture of the quadriceps muscle resulting in loss of knee flexion<sup>9,20,30</sup>. There are no controlled, prospective studies comparing the risks involved in the use of these two injection sites for immunization in infants. In Brazil, approximately 15 million doses of vaccines are administered each year to infants using the buttock as site of injection. In the present study a satisfactory serological response was observed in infants when the hepatitis B vaccine was administered into the buttock suggesting that this procedure may be considered in environments where, for cultural or operational reasons, it can facilitate the implementation of vaccination programs.

## RESUMO

### Vacina da hepatite B em lactentes: um ensaio clínico controlado, aleatorizado, comparando a administração no glúteo com a administração no vasto lateral da coxa

Diminuição significativa da resposta sorológica à vacina da hepatite B foi documentada em adultos quando a mesma é aplicada no glúteo. Entretanto, em alguns países, o glúteo ainda é bastante utilizado para aplicação intramuscular de vacinas em lactentes. Nesta faixa etária, não há estudos controlados que tenham avaliado a imunogenicidade da vacina da hepatite B quando administrada em diferentes locais. No presente estudo, 258 lactentes foram sorteados de forma aleatória para receber a vacina da hepatite B no glúteo (n = 123) ou no músculo vasto lateral da coxa (n = 135). O esquema vacinal consistiu em 3 doses da vacina da hepatite B (Engerix B<sup>®</sup>, 10 µg) aos 2, 4 e 9 meses de idade. Não se observaram diferenças significativas nas proporções de soroconversão (99,3% X 99,2%), ou na média geométrica dos títulos de anti-HBs (1.862 X 1.229 mUI/mL) entre os dois grupos. Este estudo demonstra uma resposta sorológica satisfatória em lactentes quando a vacina da hepatite B é aplicada no glúteo.

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