Anti-HBs levels among children and adolescents with complete immunization schedule against hepatitis B virus. A cross-sectional study in Blumenau, State of Santa Catarina, Brazil, 2007-2008

Níveis de anti-HBs entre crianças e adolescentes com o esquema completo de imunização contra o vírus da hepatite B. Um estudo transversal em Blumenau, Estado de Santa Catarina, 2007-2008.

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ABSTRACT

Introduction: Vaccination is the main tool for preventing hepatitis B virus (HBV) infection; however, following the completion of the vaccination series, the concentrations of anti-HBs can decline over the years and reach levels less than 10mIU/mL. The persistence of protection in these individuals is still unknown. The present study aimed to determine the anti-HBs antibody levels among children and adolescents who had received a complete vaccination course for hepatitis B. **Methods:** Antibodies against HBV surface antigen (anti-HBs) were tested in 371 individuals aged 10 to 15 years-old. **Results:** Volunteers who showed undetectable quantities of anti-HBs accounted for 10.2% of the population studied and 39.9% presented antibody titers of less than 10mIU/mL. Anti-HBs ≥ 10mIU/mL were verified in 49.9%. **Conclusions:** These results corroborate other studies indicating levels of anti-HBs below 10mIU/mL in vaccinated individuals. Additional studies are required to assess whether this indicates susceptibility to HBV infection and the need and age for booster doses.

Keywords: HBV. Vaccination. Immunity.

RESUMO

Introdução: A vacinação é o principal instrumento para prevenir a infecção pelo vírus da hepatite B. Todavia, após a conclusão da série de vacinação, as concentrações de anti-HBs podem diminuir ao longo dos anos e atingir níveis inferiores a 10mUI/mL. A persistência da proteção nestes indivíduos ainda é desconhecida. O presente estudo objetivou determinar os níveis do anticorpo anti-HBs em crianças e adolescentes que receberam o esquema completo de vacinação para a hepatite B. **Métodos:** O anticorpo para o antígeno de superfície do vírus da hepatite B (anti-HBs) foi testado em 371 indivíduos com idade entre 10-15 anos. **Resultados:** Os voluntários que apresentaram quantidades indetectáveis de anti-HBs corresponderam a 10,2% da população estudada, e 39,9% apresentaram títulos do anticorpo inferiores a 10mUI/mL. Anti-HBs ≥ 10mUI/mL foi verificado em 49,9%. **Conclusões:** Nossos resultados corroboram com outros estudos que indicam níveis de anti-HBs inferiores a 10mUI/mL em indivíduos vacinados. Estudos adicionais são necessários para avaliar se isso indica suscetibilidade à infecção pelo HBV e necessidade e idade para a dose reforço.

Palavras-chaves: HBV. Vacinação. Imunidade.

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Received in 16/12/2010
Accepted in 17/02/2011

INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus of the *Hepadnaviridae* family. It contains four open reading frames: the *S* gene (coding for the envelope proteins), the core gene (coding for the core and *e* proteins), the P gene (coding for a DNA polymerase) and the X gene (coding for a transcriptional transactivator). The envelope and core proteins are related to the viral structure, while the polymerase controls viral replication. The role of the X protein could be linked to hepatocarcinogenesis. The major proteins detected in HBV infection are the surface antigen (HBsAg), core antigen (HBcAg), and the e antigen (HBeAg). The HBsAg is an outer surface envelope protein and the HBcAg and HBeAg are both located in the nucleocapsid core protein containing the HBV genome[1-3].

The virus is not directly cytopathic and lysis of infected hepatocytes depends on the immune response of the host. Patients who develop chronic hepatitis show a pure cell-mediated immune response to the virus. If the response is particularly poor, little or no liver damage ensues and the virus continues to proliferate in the presence of normal liver function. Patients with better cell-mediated immune responses show continued hepatocellular necrosis, but the response is insufficient to clear the virus and chronic hepatitis results[2]. Chronic hepatitis caused by HBV can progress to cirrhosis and death from liver failure and chronic HBV infection is the major cause of hepatocellular carcinoma (HCC) worldwide. HBV causes 60 to 80% of HCCs worldwide[4].

In 1981, the first hepatitis B vaccine was approved in the United States. It was prepared from the plasma of HBsAg carriers and was capable of stimulating the production of antibodies against HBsAg. The current hepatitis B vaccines are not

produced from live viruses, rather they are genetically engineered and manufactured from noninfectious, recombinant DNA for HBsAg. A plasmid containing the gene that codes for HBsAg is incorporated into the DNA of *Saccharomyces cerevisiue* cells. The yeast cells are then lysed and the HBsAg is separated from the yeast components[5].

Vaccination against HBV is the most effective way of preventing infection by and the transmission of the virus[6]. In the primary three-dose course of immunization (0, 1, 6 month schedule), the first two doses usually suffice to initiate anti-HBs production and to prime the immune system for a secondary response to antigen. The third dose stimulates this secondary response, anti-HBs titers are higher than those achieved after the first two doses and the antibodies appear in the blood more rapidly. The strength of the immune response following administration of hepatitis B surface antigen (HBsAg), which is the basis of immunization against hepatitis B, has historically been assessed by measuring antibodies against HBs[7].

Hepatitis B virus vaccination at birth prevents perinatal and early childhood infection and is expected to provide protection throughout adolescence and young adulthood, when the chances of exposure to the virus are accentuated due to risky practices, including sexual activity and injectable drug use[8,9]. In Brazil, the HBV vaccine was included in the National Immunization Program in 1996. The program includes the prevention of perinatal infection, through maternal screening and prophylaxis of newborns, HBV vaccination for all children, to prevent the infection in childhood and vaccination of adolescents who were not protected and individuals belonging to risk groups. One of the goals of the Brazilian Health Ministry is the immunization of young people under 19 years of age[10]. The aim of this study was to determine the anti-HBs antibody levels among children and adolescents who had received a complete vaccination course for hepatitis B.

METHODS

Study design and studied population

A cross-sectional study was conducted to determine the anti-HBs titers among children and adolescents who had received three doses of hepatitis B vaccine.

The sample size was calculated based on the statistical formula $n \cong 4z_{\alpha}^{2} p\,q / (2ME)^2$, being z = z value of the normal curve (usually bicaudal), p = initial estimate of the proportion, q = p-complement (1-p) and ME = margin of error on the maximum tolerable parameter[11]. Considering 0.5 as the initial estimate of the proportion and the complement of p equal to 0.5, with a 95% confidence interval and 0.05 alpha error, the calculation resulted in the need for at least 384 participants.

An amostral plan was designed, aiming to reproduce the distribution of the population of children and adolescents attended by the Family and Community Health Program outpatient clinics in Blumenau, State of Santa Catarina. A list of healthcare units was requested by the Health Secretary of Blumenau, which were divided according to their location (central, east, west, north and south). The number of healthcare units and individuals from each region was determined according to the proportion of the distribution of healthcare institutions and the estimated population of the districts in each region, proposed by the Planning Secretary of Blumenau. The healthcare units were included in the study by randomized selection and the distribution of the volunteers according to healthcare unit was conducted according to the estimated population of the districts where they were located. From 10 to 30 individuals were evaluated in each of the 17 out of 40 healthcare units included in the study. The volunteers were included by randomized selection.

The inclusion of study participants was achieved according to the following criteria: children and adolescents aged between 10 and 15 years-old, female and male and the presentation of a term of free, informed consent by the child's parents or legal guardians. A total of 393 samples were collected. Twenty-two samples were excluded due to lack of data on the number of hepatitis B vaccine doses or the presence of HBV infection markers. Three hundred and seventy-one children and adolescents attended at the preselected healthcare institutions between October 2007 and August 2008 were included in the study.

Data processing and analysis

Vaccination status was checked on the vaccination certificate. A blood sample was obtained from every individual at enrolment to measure the concentration of antibodies against HBV surface antigen. The blood samples were collected by venipuncture at the healthcare institutions selected for the survey, to determine the immunological marker anti-HBs. Following separation of the serum, the samples were stored at -20°C to perform the serological tests. The analysis of blood samples was performed in the Municipal Laboratory of Blumenau, State of Santa Catarina.

Serologic testing

Anti-HBs were detected by microparticle enzyme immunoassay (MEIA) using commercial kits AxSym® (Abbott Diagnostics, Chicago, Illinois, USA). The MEIA is a variation of the principle of enzyme immunoassay (EIA) and the solid phase comprises microparticles that increase the sensitivity of the method. This solid phase EIA uses the antigens and/or antibodies adsorbed on the surface to bind to complementary analytes. The bound analyte is detected by a number of antibody-antigen reactions. At the end of the reaction, an antibody linked to an enzyme acts on a substrate and produces a fluorescent end product. The fluorescence produced by the enzymatic reaction is measured and is proportional to the amount of antibodies bound. The recombinant antigen is adsorbed on the solid microparticles of the EIA, thus quantitatively detecting the anti-HBs.

Ethical considerations

This study was approved by the Ethics Committee on Human Research of the Federal University of Santa Catarina, under protocol n 238/07, and was further approved by the Health Secretary of Blumenau.

RESULTS

A total of 371 individuals were enrolled in the study. The mean age was 12.5 years-old (±1.7), ranging between 10 and 15 years-old. **Table 1** shows the main characteristics of the group studied. Volunteers who showed no evidence of detectable anti-HBs accounted for 10.2% (38/371) of the study sample, while 39.9% (148/371) presented antibody quantities of less than 10mIU/mL. Anti-HBs ≥ 10mIU/mL was documented in 49.9% (185/371). The serological results of the population studied are displayed in **Table 2**.

TABLE 1- Demographic characteristics of study population.

	Number	Percentage
Sex		
male	170	45.8
female	201	54.2
Age at enrolment (years)		
10-11	101	27.2
12-13	148	39.9
14-15	122	32.9
Residential location (region)		
Central	18	4.8
East	49	13.2
North	170	45.8
West	75	20.2
South	59	15.9

TABLE 2 - Anti-HBs antibody titers in the studied population.

Anti-HBs titers	Number	Percentage
Undetectable	38	10.2
< 10mIU/mL	148	39.9
≥ 10mIU/mL	185	49.9
Total	371	100.0

DISCUSSION

Worldwide, the hepatitis B immunization program has demonstrated a reduction in the rates of infection. In Thailand, the seroprevalence of HBsAg in children under 18 years of age decreased from 2.3% in 1999 to 1.4% in 2004, and a decline in the prevalence of HBsAg among adults was also observed[12]. In the United States, the incidence of acute hepatitis B decreased 94% among children and adolescents from 1990 to 2004, following the implantation of the vaccination program[8]. The positive impact of the vaccination program against HBV in Saudi Arabia is evident from the incidence of hepatocellular carcinoma, which fell from 2.6 in 1994 to 1.9 per 100,000 inhabitants in 2001[13].

This analysis of the immunity against HBV among children and adolescents aged 10 to 15 years-old who received hepatitis B vaccine corroborates previous studies indicating levels of anti-HBs less than 10mIU/mL in vaccinated individuals[12,14,15]. Half of the study participants showed antibodies against hepatitis B surface antigen below 10mIU/mL. These individuals may be hyporesponsive to the immunization and their antibodies might rapidly wane over time. However, loss of antibody may not imply loss of protection, since the incubation period of HBV could allow time for the immunological memory to protect them against acute disease or the development of chronic carriage[15].

Following the completion of the vaccination series, the concentrations of anti-HBs may decline over the years and can reach levels less than 10mIU/mL. However, despite the low concentrations of anti-HBs, HBV infection is uncommon in individuals responsive to the primary vaccination series. The immune memory may persist even after the decline of anti-HBs concentrations and protection against HBV infection is provided by specific memory T and B lymphocytes generated in response to the primary vaccination series[7,9,15-18]. An immune memory response (or anamnestic response) followed by a booster dose is evidence that the immune memory cells remain functional and could protect against HBV infection[9]. Some immunogenicity studies have assessed response to a booster vaccination and verified that 51 to 97% of those vaccinated showed anamnestic responses[9,14,15]. One study showed an anamnestic response to the booster dose administered more than 20 years after the primary vaccination in 95.8% of subjects, suggesting a strong persisting immune memory[19]. Two other studies have reported that protection provided by HBV vaccine persists for at least two decades in the great majority of vaccinated individuals[20,21].

Results from this study provide information regarding immunity in a teenage population, when individuals may be at increased behavioral risk of exposure to HBV. One similar study conducted among children and adolescents aged 10 to 16 years-old in the metropolitan area of Florianópolis, State of Santa Catarina, reported that 31.5% showed anti-HBs levels less than 10mIU/mL and 9.6% did not present detectable antibody titers. However, since most of the participants did not present their vaccination cards, it is not known whether individuals with undetectable antibody titers represent non-vaccinated individuals, vaccine primary non-responders or whether their antibody titers declined over time[22].

The main limitation of this study was the failure to collect data concerning the age of vaccination, which could have yielded an assessment of the relation between vaccination age and test age. This may influence the anti-HBs reactivity.

Several factors can influence the results of hepatitis B vaccine trials, such as differences in vaccine formulation and production, site of vaccine administration, concomitant immunization with other vaccines and age at vaccination[23]. One report has revealed that students vaccinated at preschool age present higher anti-HBs positive rates than those vaccinated at birth, which implies that the production and persistence of anti-HBs may be dependent on the time of vaccination, such that the optimal vaccination time for HBV vaccine in children may be re-evaluated[24].

The HBsAg seroprevalence is still higher in adolescents than in children[25], what implies that there were some individuals whose anti-HBs decreased from their childhood to adolescent period and became susceptible to HBV infection[24]. One report also revealed that the persistence of higher anti-HBs levels was associated with vaccination at older age[26]. On the other hand, studies have shown low acceptance of HBV vaccine among adolescents, especially those of low-income[27-29]. Furthermore, in areas of high prevalence of HBV infection, the predominant mode of transmission is perinatal and the disease is transmitted vertically during early childhood from the mother to the infant[30]. Thus, the lower vaccination coverage due to lower adhesion to the vaccination series and the carrier rates of HBsAg in newborns associated with mother-to-child transmission must also be considered. The maintenance of mandatory vaccination of newborns is important to reduce HBV perinatal transmission, particularly in hyperendemic areas. However, the necessity of a booster dose to sustain immunity into adolescence and adulthood when the risk of infection increases should be evaluated.

In conclusion, analysis of the results obtained corroborates other studies indicating high levels of anti-HBs less than 10mIU/mL in vaccinated individuals. However, the duration of protection following hepatitis B vaccination in these individuals remains unknown. Although HBV infection at birth or in early childhood

is associated with a high risk of chronic infection development and HCC[8], the continued success of the hepatitis B vaccination program is dependent on the capacity of the vaccine to induce lasting protection during adolescence and early adulthood. Additional studies are required to assess whether antibody levels less than 10mIU/mL in vaccinated individuals indicate susceptibility to HBV infection and the necessity and age for booster doses. Assessment of the presence of memory cells may provide a useful starting point for determining the long-term immunity following vaccination.

ACKNOWLEDGMENTS

The authors would like to thank the volunteers who participated in the study, the Health Secretary of Blumenau and healthcare workers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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