

# Seroprevalence of hepatitis B and hepatitis C markers among children and adolescents in the south brazilian region - metropolitan area of Florianópolis, Santa Catarina

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## ABSTRACT

Hepatitis B and C are important causes of morbidity and mortality worldwide. In Brazil, according to the Ministry of Health, about 15% of population is infected by hepatitis B virus (HBV) and less than 1% by hepatitis C virus (HCV). Nevertheless, the age-specific prevalence of HBV and HCV markers remains unknown. This study aimed to determine the seroprevalence of HBV and HCV markers of infection and immunity in children and adolescents between 10 to 16 years old who live in the metropolitan area of Florianópolis, state of Santa Catarina, South of Brazil. Three hundred and eighty four individuals were enrolled in this study. Serological markers for HBV and HCV (HBsAg, total anti-HBc, anti-HBc IgM, anti-HBs and anti-HCV) were determined through Microparticle Enzyme Immunosorbant Assay (MEIA) - AxSYM System® - by using commercial diagnostic kits (Abbott Laboratories, Abbott Park, Illinois, USA). All 384 adolescents (100%) were negative for HBsAg and anti-HBc IgM. Only two (0.52%) were positive for total anti-HBc. Among the studied individuals, 226 (58.85%) presented titers of anti-HBs  $\geq$  10.0mIU/mL, 121 (31.51%) presented anti-HBs < 10.0mIU/mL, and 37 (9.64%) did not present titers of anti-HBs. Regarding to anti-HCV, all 384 adolescents (100%) presented negative results for this marker. In conclusion, this study showed a low prevalence of HBV and HCV infections. In addition, it was verified a great number of children and adolescents (89.84%) who were positive for the immunity marker anti-HBs, implying that the National Immunization Program Protocol for hepatitis B has been effective in the studied region.

**Keywords:** HBV, HCV, HBsAg, anti-HBs, anti-HBc, anti-HCV, seroprevalence.

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## INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the most common causes of liver disease worldwide.<sup>1</sup> Both viruses can be transmitted parenterally, sexually and perinatally, with perinatal and sexual transmission being more common for HBV<sup>2,3</sup> than for HCV.<sup>4</sup> Despite progress on prophylaxis, diagnostic and treatment of these infections, they still are one of the major causes of chronic liver disease, such cirrhosis or hepatocellular carcinoma.<sup>5,6</sup>

According to the World Health Organization (WHO) it is estimated that, currently, more than 2 billion of the global population had been infected with HBV. Out of these, approximately 360 million are chronically infected and at risk of serious illness and death from liver cirrhosis and liver cancer, diseases that are estimated to cause 500,000 to 700,000 deaths each year worldwide.<sup>7</sup>

Also according to WHO estimates, about 170 million people, 3% of the world's population, are infected with HCV and are at risk of developing liver cirrhosis and/or liver cancer.<sup>8</sup>

In Brazil, the Ministry of Health estimates that 15% of the population have been in contact with HBV and not less than 1% of the population has chronic disease.<sup>9</sup> However, there are not many studies revealing the real prevalence of HCV among Brazilians.<sup>10</sup> Data obtained from blood donor banks, in 2002, showed a different distribution of the illness among Brazilian regions. In the North region was found an incidence of 0.62%, in the Northeast 0.55%, in the West-Center 0.28%, in the Southeast 0.43% and in the South 0.46%.<sup>11</sup>

Due to Brazilian's territorial extension and its economic and cultural differences, hepatitis B and C infection rates are different in each region.<sup>11</sup> Epidemiologic studies regarding to the distribution of these illnesses in Brazil are

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uncommon. In general, such studies focus the incidence rather than prevalence, like in the study of Charles *et al.*, which demonstrated the incidence of hepatitis B in the South region of Brazil from the year of 1997 to 1999.<sup>12</sup>

The main reason for the predominance of incidence studies rest in the way how the data are collected. The term incidence refers to the number of new cases of infection, emerged in a certain population, at determined interval of time. Therefore, incidence studies do not need deal with population directly, because this information is obtained from health public organization, like Epidemiologic Vigilance. On the other hand, the prevalence is established by the total number of positive cases of infection, in a certain population at specific time. And to get the prevalence rates, tests with population are needed. In fact, the difficulty in gathering the needful number of blood samples among the ordinary population is the main reason for this lack of prevalence studies.<sup>13</sup> So, these studies are almost always performed in specific groups, like blood donors<sup>14-17</sup> or HIV-seropositive patients.<sup>18,19</sup>

Hepatitis B and C are important diseases and have serious consequences. Prevention is the best alternative to control their spreading. For hepatitis C there is not a vaccine, and its prevention is reached only by educational programs, but for hepatitis B vaccination exists and is essential. The prevention of hepatitis B by vaccination is one of the most efficient tools to avoid the transmission of the virus.

In 1989, the vaccination against hepatitis B began in some regions of Brazil, for specific groups. Some years later, it was enlarged to more regions and to children younger than one year old, as well as to high risk populations. Afterwards, the coverage of the vaccine was amplified for health students and militaries, and then, for adolescents up to 15 years old in some Federal States. Regarding specifically to Santa Catarina state, in 1993 the vaccination was recommended to children younger than four years old, and in 1996, to children up to fifteen years old. In 2001 the immunization was extended to whole Brazilian territory, comprehending the population up to nineteen years old.<sup>11</sup>

This study aimed to determine the prevalence of HBV and HCV infections in children and adolescents between 10 and 16 years old who live in the metropolitan area of Florianópolis, state of Santa Catarina, in South of Brazil. For this purpose, serological assays of hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (total anti-HBc and anti-HBc IgM), and hepatitis C antibody (anti-HCV) were performed. Moreover, one pretends to determine the prevalence of the marker for immunity to hepatitis B in the same population, using antibody to hepatitis B surface antigen (anti-HBs), allowing one evaluates the effectiveness of the National Immunization Program Protocol for hepatitis B.

## MATERIAL AND METHODS

The studied group was formed by children and adolescents between 10 to 16 years old, residents in the metropolitan area of Florianópolis, whose blood was tested, re-

gardless the reason, in the Clinical Analysis Laboratories of the same region. An additional amount of blood was drawn from the same venous puncture site used to collect the original sample for the testing assigned to the patient at the Clinical Analysis Laboratory. The blood samples were obtained between May 2007 and August 2008 in 14 Clinical Analysis Laboratories distributed around the region of interest.

The study was approved by the Ethical Committees of the Federal University of Santa Catarina (Project nº 351/2006). A written informed consent was obtained from the legal responsible persons of each participant since all subjects were under the legal majority age (18 years old).

The minimum number of samples  $n$  was defined by<sup>20</sup>

$$n = \frac{z \alpha^2 pq}{E^2}$$

where  $\alpha$  is the confidence interval,  $z$  is the corresponding value of  $\alpha$  at normal curve,  $p$  is the probability of finding the studied phenomenon,  $q$  ( $q = 1-p$ ) is the complementary part of probability, and  $E$  is the admissible error. In this study a confidence interval of 95% was adopted. Thus,  $\alpha = 0.05$  and  $z = 1.96$ . Unknown prevalence markers at studied population makes  $p$  equals 50% ( $p = 0.5$ ) and, consequently,  $q$  equals 0.5. The admissible error was bounded in 5% ( $E = 0.05$ ). Using such criteria the minimum sample number was defined as 384, number reached at the end of the study.

The collected blood samples were centrifuged to separate the serum, which was forwarded then to laboratorial analysis. Serological markers for HBV and HCV (HBsAg, total anti-HBc, anti-HBc IgM, anti-HBs and anti-HCV) were determined through Microparticle Enzyme Immunosorbant Assay (MEIA) - AxSYM System®, by using commercial diagnostic kits (Abbott Laboratories, Abbott Park, Illinois, USA) and following the instructions provided by the producer. All laboratory assays were performed at the Clinical Analysis Laboratory of University Hospital of Federal University of Santa Catarina, in Florianópolis. Then, prevalence of each serological marker was established according to sex.

## RESULTS

The main characteristics of the 384 children and adolescents are showed at Table 1. All 384 adolescents (100%) were negative for HBsAg (Table 2) and anti-HBc IgM (Table 3). Only two adolescents (0.52%) were positive for total anti-HBc (Table 4). Among the studied individuals, 226 (58.85%)

**Table 1. Demographic study population**

Sex	Adolescents (n = 384)
male	163 (42.45%)
female	221 (57.55%)
Age at enrollment (years; mean ± D)	12.59 ± 2.02
<b>Residential location</b>	
Florianópolis	241 (62.76%)
Metropolitan area	143 (37.24%)

**Table 3. Prevalence of anti-HBc IgM according to sex**

Sex	anti-HBc IgM (+)	anti-HBc IgM (-)
male	0 (0%)	163 (42.45%)
female	0 (0%)	221 (57.55%)
<b>Total</b>	<b>0 (0%)</b>	<b>384 (100%)</b>

\*Anti-HBc IgM: antibody (immunoglobulin M) to hepatitis B core antigen

**Table 5. Prevalence of anti-HBs according to sex**

Sex	anti-HBs = 0.0 mIU/mL	0.0 > anti-HBs < 10.0mIU/mL	anti-HBs ≥ 10.0mIU/mL	Total
male	17 (4.43%)	50 (13.02%)	96 (25%)	163 (42.45%)
female	20 (5.21%)	71 (18.49%)	130 (33.85%)	221 (57.55%)
<b>Total</b>	<b>37 (9.64%)</b>	<b>121 (31.51%)</b>	<b>226 (58.85%)</b>	<b>384 (100%)</b>

\* Anti-HBs: antibody to hepatitis B surface antigen

**Table 6. Comparison of HBV markers prevalence according to sex**

Sex	Total anti-HBc (+) anti-HBc IgM (-) HBsAg (-) anti-HBs (+)	Total anti-HBc (-) anti-HBc IgM (-) HBsAg (-) anti-HBs (-)	Total anti-HBc (-) anti-HBc IgM (-) HBsAg (-) anti-HBs (+)	Total
male	2 (0.52%)	17 (4.43%)	144 (37.50%)	163 (42.45%)
female	0 (0%)	20 (5.21%)	201 (52.34%)	221 (57.55%)
<b>Total</b>	<b>2 (0.52%)</b>	<b>37 (9.64%)</b>	<b>345 (89.84%)</b>	<b>384 (100%)</b>

\*Total anti-HBc: antibodies (immunoglobulin M and G) to hepatitis B core antigen; \*anti-HBc IgM: antibody (immunoglobulin M) to hepatitis B core antigen; \*HBsAg: hepatitis B surface antigen; \*anti-HBs: antibody to hepatitis B surface antigen

presented titers of anti HBs ≥ 10.0mIU/mL, 121 (31.51%) presented anti HBs < 10.0mIU/mL, and 37 (9.64%) did not present titers of anti-HBs (Table 5). Thirty seven individuals were negative for all HBV markers studied, 345 (89.84%) were positive only for anti-HBs and 2 (0.52%) were positive for anti-HBs and total anti-HBc (Table 6). Regarding to anti-HCV, all individuals presented negative results for this marker (Table 7).

**Table 2. Prevalence of HBsAg according to sex**

Sex	HBsAg (+)	HBsAg (-)
male	0 (0%)	163 (42.45%)
female	0 (0%)	221 (57.55%)
<b>Total</b>	<b>0 (0%)</b>	<b>384 (100%)</b>

\*HBsAg: hepatitis B surface antigen

**Table 4. Prevalence of total anti-HBc according to sex**

Sex	total anti-HBc (+)	total anti-HBc (-)	Total
male	2 (0.52%)	161 (41.93%)	163 (42.45%)
female	0 (0%)	221 (57.55%)	221 (57.55%)
<b>Total</b>	<b>2 (0.52%)</b>	<b>382 (99.48%)</b>	<b>384 (100%)</b>

\*Total anti-HBc: antibodies (immunoglobulin M and G) to hepatitis B core antigen

**Table 7. Prevalence of anti-HCV according to sex**

Sex	anti-HCV (+)	anti-HCV (-)
male	0 (0%)	163 (42.45%)
female	0 (0%)	221 (57.55%)
<b>Total</b>	<b>0 (0%)</b>	<b>384 (100%)</b>

\*Anti-HCV: hepatitis C antibody

## DISCUSSION

According to the Ministry of Health, the South of Brazil is considered a low prevalence region of hepatitis B infection (less than 2% of population is positive for HBsAg). Nevertheless, there are exception for the west region of Santa Catarina, classified as a moderate prevalence region (between 2% and 7% of HBsAg positive) and the west of Paraná, which presents high prevalence levels (higher than 7% of HBsAg positive) of hepatitis B infection.<sup>11</sup> The few prevalence studies in Brazil have showed low numbers for its infection markers.<sup>13-15,17</sup> Miranda *et al.*<sup>13</sup> established prevalence of hepatitis B serological markers among 632 individuals submitted to blood tests in health centers of Ribeirão Preto, São Paulo. Such data were collected between 1994 and 1995. They found 0.3% of prevalence for HBsAg, specifically found in two individuals aged more than 60 years. Regarding to anti-HBc, it was found a prevalence of 13.9%. However, there was no case of positive anti-HBc in the age-group of 10 to 19 years old.

Furthermore, prevalence of HBV infection markers has been decreasing in last years.<sup>14,17,21</sup> Silveira *et al.*,<sup>21</sup> in a study of hepatitis B seroprevalence in Latin America, carried out in 1994 to 1997, described a wide range of seroprevalences in the different regions of Brazil, with a high rate in Manaus (21%), followed by Porto Alegre (7.5%), Rio de Janeiro (5.5%), and Fortaleza (1.2%). Rosini *et al.*<sup>17</sup> estimated prevalence of serological markers for HBV infection among blood donors in Santa Catarina in the years of 1999, 2000 and 2001. They found HBsAg prevalence of 0.98% (1999), 0.84% (2000) and 0.64% (2001), and anti-HBc prevalence of 8.83% (1999), 7.09% (2000) and 5.35% (2001).

Recently, the Brazilian Ministry of Health, with some Universities' help, had realized a seroprevalence study about viral hepatitis, in the capital of each state. Some results were already presented.<sup>22</sup> According to this inquiry, in the South region, an anti-HBc prevalence of 1.18% was found for adolescents between 10 and 19 years old, and 11.2% for adults between 20 and 69 years old. For anti-HBs, the prevalence were 0.12% for adolescents, and 0.5% for adults.

The results represent prevalence of hepatitis B in urban population, and in this case attention is needed, since it is fact that higher prevalence usually is found in countryside, especially Amazon region, south of Espírito Santo, west of Paraná and Santa Catarina, regions not contemplated at the study.<sup>22</sup>

In the present study, a very low prevalence of HBV infection was found, like in the studies presented before. Only two individuals (0.52%) presented an infection marker of hepatitis B (total anti-HBc) positive. Furthermore, they presented negative results for anti-HBc IgM and HBsAg. The anti-HBc IgM negative means that contact was not recent, whereas the HBsAg negative means that virus was depurated of the organism. Such results characterize a past cured infection.

Regarding to immunity, the majority of the studied population in this work (58.85%) presented titers of anti-HBs  $\geq 10.0$  mIU/mL, criterion adopted to establish protective concentrations.<sup>23-25</sup> Moreover, 31.51% of the individuals presented anti HBs  $< 10.0$  mIU/mL. The remainder 9.64% did not present titers of anti-HBs.

The anti-HBs is responsible for the immunity against HBV. In addition to cured infections, it is generally acquired through vaccination. Primary vaccination with a three-dose series of hepatitis B vaccine results in seroprotection (defined as the development of anti-HBs at a concentration greater than 10 mIU/mL) in  $> 95\%$  of vaccinated infants and children.<sup>23-25</sup> However, following completion of the primary series, anti-HBs concentrations decline and may fall below 10.0 mIU/mL after several years. Despite the criterion adopted to establish protective concentrations of anti-HBs ( $\geq 10.0$  mIU/mL), recent studies argue the immunologic memory would be capable of preventing chronic or symptomatic infections even after anti-HBs declines to undetectable concentrations in vaccinated ones.<sup>26-34</sup>

Based on previous discussion, one concludes that all individuals in this study, who presented positive titers of anti HBs and were negative for infection markers (89.84%), may have been vaccinated (Table 6). However, the sample was not random and is not population-based, so it could not be representative of the population. Concerning to Brazilian Immunization Program, the vaccination coverage for hepatitis B in the state of Santa Catarina has reached 95% in the last years, for infants under one year old. On the other hand, the range of vaccination coverage for children and adolescents is not available for this population.<sup>35</sup>

The Secretariat of Epidemiologic Vigilance, department subordinate to the Brazilian Ministry of Health, does not have a database referent to the vaccination program. Pertinent information such as who was vaccinated, whether the vaccination schedule was completed and when it was accomplished can be obtained just through vaccination cards. Since the majority of the adolescents enrolled in this study did not present the vaccination cards, it is not know if individuals without titers of anti-HBs (9.64%) represent not-vaccinated individuals, if they were vaccine primary nonresponders or if their antibodies titers declined over time. Even though the vaccination cards were available, it would not be possible to have sure about this information, because Secretariat of Health does not have control in the expedition of these cards, and it is common people have more than one.

On the other hand, the prevalence rates found in the present study (Table 5) are very similar to those showed in Italy,<sup>31</sup> in which among 1,212 vaccinated children (mean age 10.9 years), 64% presented titers of anti-HBs  $\geq 10.0$  mIU/mL, 27% presented antibody titers less than 10.0 mIU/mL, and 9% presented undetectable amounts. Regarding to



infection markers, only one child presented anti-HBc positive in the Italian study,<sup>31</sup> compared to two found in ours. In both studies, there was no case of HBsAg positive.

Regarding to HCV infection, in the present study, all 384 adolescents (100%) were negative for this marker (anti-HCV), which means none infection case in the studied population. Such result was not surprising due to the HCV modes of transmission and the age-group of the studied population. In fact, one of the few Brazilian's studies about hepatitis C prevalence, carried out in São Paulo, demonstrated an estimated HCV prevalence of 1.42%. This specific infection occurred more frequently among adults 30 years of age or older, with the prevalence reaching a peak of 3.80% among the group aged 50 to 59 years.<sup>36</sup> In addition, Rosini *et al.*<sup>17</sup> estimated the anti-HCV prevalence among blood donors in Santa Catarina, in the years of 1999, 2000 and 2001. They found a prevalence of 0.41% in Florianópolis region; number a little bit higher than found in the state of Santa Catarina as a whole (0.35%).

The lack of data relative to child and adolescents in Brazil makes difficult a deep comparison of the results obtained in this study. Most of studies deal with specific groups. The prevalence found in studies with blood donors, for example, refers to an age-group higher than the object of the present study, since the minimum age for blood donor is 18 years old. On the other hand, HIV seropositive groups are not good representatives for a population in general because they refer to a group of individuals with a high risk of HBV and HCV infection due to the common modes of transmission between such diseases. Hence, more studies with children and adolescents are needed to confirm the results observed in the present study.

However, the recent Ministry of Health's inquiry brings some information regarding to anti-HCV. A prevalence of 1.63% for adolescents between 10 and 19 years old, and of 1.98% for adults between 20 and 69 years old were found. For HCV, there are not studies revealing differences on prevalence in countryside compared to cities located at big urban centers.<sup>24</sup>

Despite the uncertainties, the results observed in this study reveal a good perspective for the future. A low prevalence of HBV and HCV infection and a high prevalence of HBV immunity among children and adolescents show progress towards the control of hepatitis B and C infections in Brazil. However, it is important to keep or even improve educational programs, especially because there is not a vaccine for the HCV prevention. As a matter of fact, diseases which have the same mode of transmission, like hepatitis B, hepatitis C and AIDS, could share the same prevention campaigns.

## CONCLUSION

In conclusion, this study showed a low seroprevalence of HBV and HCV infections among children and adolescents in the metropolitan area of Florianópolis, Santa Catarina, South of Brazil. In addition, a large number of individuals

presented titers of the seroprotection marker anti-HBs, implying that National Immunization Program Protocol for hepatitis B has been effective in the studied region. Besides low prevalence of HBV and HCV infections, prevention campaigns are important to remind children and adolescents how these infections can be prevented, mainly HCV, which does not have a vaccine for its prevention.

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