

Seroprevalence of hepatitis B virus infection and seroconversion to anti-HBsAg in laboratory staff in Goiânia, Goiás

Soroprevalência da infecção pelo vírus da hepatite B e soroconversão para anti-HBsAg em profissionais de laboratório em Goiânia, Goiás

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ABSTRACT

Were analyzed 648 serum samples from laboratory staff in Goiânia, Goiás aiming detection of three serological markers of HBV: HBsAg, anti-HBsAg and anti-HBcAg. The HBsAg and anti-HBcAg positive samples were also analyzed for HBeAg, anti-HBeAg and anti-HBcAgIgM markers. HBV infection rate of 24.1% was observed and, from them, 0.7% were positive for HBsAg. Viral DNA was detected by PCR in two HBsAg positive samples. A vaccination index of 74.5% and a global index of 89.9% of serological response to vaccination were observed. The direct work with biological fluids as well as cleaning workers represented significant risks for acquisition of HBV infection. The data from the present study showed an increase of the vaccination index among laboratory staff but the rates of HBV infection did not change through the years in the region.

Key-words: Hepatitis B. Seroprevalence. Vaccination. Laboratory staff.

RESUMO

Neste estudo, foram analisadas 648 amostras de soro, provenientes de profissionais de laboratório de Goiânia-Goiás, visando a detecção de três marcadores sorológicos do VHB: HBsAg, anti-HBsAg e anti-HBcAg. As amostras HBsAg e anti-HBcAg positivas foram também analisadas para os marcadores HBeAg, anti-HBeAg e anti-HBcAgIgM. Foi observado um percentual de soropositividade para infecção pelo VHB de 24,1% sendo que 0,7% destes foram positivos para o HBsAg. O DNA viral foi detectado por PCR nas duas amostras HBsAg positivas. Dos 648 profissionais, 74,5% relataram vacinação para o VHB e, destes, 89,9% apresentaram soroconversão. Trabalho direto com fluidos biológicos bem como trabalho em serviços gerais representaram risco significativo para aquisição da infecção pelo VHB. Os resultados do estudo mostram que embora tenha havido um aumento no índice de vacinação entre os trabalhadores de laboratório, o índice de infecção pelo VHB manteve-se inalterado em relação ao tempo na região.

Palavras-chaves: Hepatite B. Soroprevalência. Vacinação. Profissionais de laboratório.

Hepatitis B virus (HBV) is one of agents of human liver diseases, including acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma³. The disease is a great health problem worldwide, with an estimate of 350 million people chronically infected⁶.

Health-care workers (HCW) are at risk for occupational exposure to blood-borne pathogens, including HBV. The annual number of occupational infections has decreased since hepatitis B vaccine became available in 1982. Nevertheless, in the United States,

approximately 800 health-care workers become infected with HBV each year following occupational exposure⁴. In Goiânia, Goiás, a vaccination program directed at immunization of people at high risk for acquiring HBV, such as HCW, was introduced in 1992.

Included in the HCW category are laboratory professionals who were the subject of this study. There are few studies considering this population and these showed a seroprevalence rate for the virus that varies from 16.4% to 23.3%^{2 5 8 15}.

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In our region, Goiânia, Goiás, studies about HBV seroprevalence in different populations have been developed, including hospital and hemodialysis staff^{2 11}. However, there are no studies regarding the seroprevalence of infection and status of vaccination for HBV in laboratory staff. In this way, this study presents the index of serological markers for HBV, the detection of viral DNA, as well as the index of professionals vaccinated against HBV and the response to vaccine.

MATERIAL AND METHODS

Population studied. In 2000, the number of laboratory professionals in Goiânia was estimated at 3000 people (Ministério do Trabalho e Emprego, Brazil). In this way, 600 professionals would be enough to detect a seroprevalence rate of infection of 22% with an accuracy of 3%. Also, in the period under study there were 150 private and 20 public laboratories in Goiânia (Conselho Regional de Biomedicina, Conselho Regional de Farmácia, Sociedade Brasileira de Análises Clínicas e Vigilância Epidemiológica do Município de Goiânia), which 30 private and 10 public laboratories were chosen at random to participate in the study. All staff involved in the study agreed with the procedure (written consent) and filled out a questionnaire requesting personal data, information about risk factors associated with hepatitis transmission, as well as vaccination against HBV. The collection of blood samples was performed between November, 2000 and June, 2001. A second blood sample was collected for follow-up from individuals who tested positive for HBsAg. This study was approved by the Ethics Committee of the Federal University of Goiás (n° 2200000167).

Detection of HBV serological markers. The blood samples were analyzed for HBsAg, anti-HBsAg and anti-HBcAg. Samples which tested positive for HBsAg or only for anti-HBcAg were also tested for anti-HBcAgIgM, HBeAg and anti-HBeAg. These procedures were performed by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Organon Teknika-Hepanostika, Boxtel, The Netherlands and Bio-Rad, France). The samples were considered positive or negative when optic density was respectively 10% above or below of the cut off value, calculated as recommended by the manufacturer.

Calculation of the HBV seroprevalence infection rate included professionals who were not vaccinated, as well as those who were vaccinated but presented other infection markers besides anti-HBsAg.

Viral DNA detection. The positive samples for HBsAg and/or anti-HBcAg were analyzed to detect viral DNA using PCR methodology. The viral DNA was extracted from serum and amplified in according to the description¹³. In the first amplification, 5 pairs of primers designed from conserved regions of the viral genome (PS1-PS2, X1-X2, C1-C2, C1-PS2 and PS1-S2), were used. The negative samples were submitted to a second amplification (semi-nested PCR), using primers PS1-PS2 and PS4-S2^{9 13}. The PCR procedure utilized is able to detect 100 copies per genome (Gomes, S.A.- personal communication).

Seropositivity to vaccine. For this analysis, vaccinated staff who did not present any serological markers for viral infection

were considered. Only individuals that tested positive for only anti-HBsAg were considered seropositive to the vaccine.

Statistical analysis. Data were analyzed using the EPINFO 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, EUA, 1997) statistical software package. A chi-square test, chi-square for trend tests and Fisher's exact test with 95% confidence intervals were utilized when necessary. Odds ratio (OR) also was utilized for evaluation of HBV risk factors.

RESULTS

Population characteristics. From 648 staff members, 496 were female and 152 male, the ranging in age was from 18 to 60 years and 38.1% were between 26 and 35 years. Of these, 389 were from private laboratories.

Seroprevalence rates for HBV. From 295 individuals analyzed, 71 (24.1%) presented serological markers for HBV: Two were positive for HBsAg/anti-HBcAg, six for anti-HBcAg alone and sixty-three for anti-HBcAg /anti-HBsAg (Table 1). It was observed that of two individuals who tested positive for HBsAg, one tested positive for the same serological markers (HBsAg/total anti-HBcAg) in both blood samples and the other only for HBsAg in the first sample and for HBsAg/anti-HBcAg in the second one. Ten staff members who reported no vaccination tested positive for anti-HBsAg alone.

Multivariate analysis for HBV infection in relation to risk factors showed statistical significance for professionals that worked directly with biological fluids and in cleaning (Table 2).

Table 1 - Seroprevalence of HBV infection in 295 laboratory workers in Goiânia, Goiás, considering serological markers.

Serological markers	Positive		CI* 95%
	n°	%	
HBsAg / anti-HBcAg total	2	0.7	0.11 - 2.7
Anti-HBcAg total	6	2.0	0.8 - 4.5
Anti-HBcAg total/anti-HBsAg	63	21.4	16.8 - 26.5
Total	71	24.1	19.3 - 29.4

*CI=Confidence Interval ; Ten staff members reported no vaccination but tested positive for anti-HBs only were not included in this analysis.

Table 2 - Multivariate analyses for risk factors of HBV infection in laboratory workers in Goiânia, Goiás.

Characteristics	OR (CI 95%)	OR adjusted ^a (CI 95%)
Age (years)		
15-25	1	1
26-35	4.37 (1.57-12.77)	2.90 (0.95-8.88)
36-45	6.19 (2.18-18.46)	2.54 (0.74-8.68)
46-60	11.15 (3.64-35.76)	3.21 (0.85-12.09)
Laboratory type		
private	1	1
public	2.46 (1.38-4.41)	1.65 (0.85-3.22)
Years of service		
0 - <1	1	1
1 - <5	1.47 (0.54-4.13)	1.26 (0.45-3.52)
5 - <10	4.25 (1.54-11.96)	2.37 (0.81-6.94)
>10	6.12 (2.32-16.6)	3.02 (0.94-9.70)
Type of work		
administrative	1	1
with biological fluids	6.84 (2.47-20.41)	3.04 (1.23-9.45)
cleaning	12.83 (3.86-5.27)	7.33 (2.17-24.72)

^aOdds Ratio adjusted by gender, age, laboratory type, length of service and type of work.

Detection of viral DNA. Positivity for HBV-DNA was observed in the two HBsAg positive samples.

Vaccination rates. 623 of 648 staff provided information about vaccination and of them 464 (74.5%) had been vaccinated. A smaller vaccination rate was observed among individuals with low educational levels, public laboratory workers, administrative and cleaning workers, as well as workers less than one year on the job ($p < 0.05$).

Seropositivity to vaccine. A seropositivity rate of 89.9% to the vaccine for individuals that received three doses of HBV vaccine was observed. The male individuals and those of 46-60 years of age or who had low educational levels had lower seropositivity to the vaccine (Table 3 and 4).

Table 3 - Percentage distribution of seropositivity to the HBV vaccine in 415 laboratory workers in Goiânia, Goiás.

Characteristics	Seropositivity to vaccine		p
	positive n°	vaccinated %	
Gender			
F	276/323	85.4	p<0.05
M	67/92	72.8	
Age ^a (years)			
15-25	86/103	83.5	p<0.05
26-35	148/168	88.1	
36-45	74/96	77.1	
46-60	32/44	72.1	
Level of education ^b			
university	173/198	87.4	p<0.05
secondary	137/176	77.8	
none/Elementary	32/40	80.0	
Laboratory type			
private	192/238	80.7	p>0.05
public	151/177	85.3	

Positive/vaccinated = number of individuals with positive samples for anti-HBsAg/total vaccinated individual. ^a 4 individuals without information about age; ^b 1 individual without information about level educational.

Table 4 - Percentage distribution of seropositivity to the HBV vaccine in 415 laboratory workers in Goiânia, Goiás.

Characteristics	Seropositivity to vaccine		p
	positive n°	vaccinated %	
Years of service ^a			
0 - <1	45/55	81.8	p>0.05
1 - <5	127/153	83.0	
5 - <10	87/98	88.8	
>10	73/94	77.6	
Type of work			
administrative	39/53	73.6	p>0.05
with biological fluids	271/324	83.6	
cleaning	33/38	86.8	
Number of vaccine doses			
1	14/34	41.2	p<0.001
2	57/76	75.0	
3	231/257	89.9	
NI ^b	41/47	87.2	

Positive/vaccinated = number of individuals with positive samples for anti-HBsAg/total vaccinated individual. ^a 15 individuals without information about length of laboratory service; ^b individuals without information about number of vaccine doses.

DISCUSSION

In this study, the rate of seroprevalence for infection observed for HBV among laboratory staff is in agreement with other studies^{2 5 8 15}. Direct contact with biological fluids as well as laboratory cleaning constituted risk factors for HBV infection. This result reinforces data from other studies and the concept that blood and biological fluids are relevant factors for acquisition of infection for this type of population¹⁹.

The follow up of two staff tested positive for HBsAg, showed that one had HBsAg for more than one year and was thus considered a chronic carrier. For the other staff member the antiHBcAg was present only in the second sample and in whole these data reaffirm the circulation of the virus in this population.

In the present study, no positivity was observed for viral DNA in positive samples for anti-HBcAg only which is in agreement with a study performing by Largura et al¹⁰. However, study shows that even in intermittent viremia the viral DNA can be detected when ultrasensitive nucleic acid amplification testing (3.8 IU/mL) is utilized⁷. On the other hand, in Goiânia, Goiás, 3.6% of positive rate for viral DNA in this type of sample was observed utilizing the same procedure¹⁴. This difference may be the result of the type of population studied since in this study the participants were patients with clinic suspect of hepatitis. Positive samples for viral DNA were also positive for HBsAg and were from the second blood samples. This data suggest that more than one sample of clinical specimens may be necessary before considering the results for HBV-DNA.

The percentage of staff who had received HBV vaccine was 74.5% and is higher than the percentage reported in other studies of health-care workers in 1989 and 1998 in the region, in which vaccination rates of 3% and 59.2%, respectively were reported^{2 11}.

Seropositive rate of 89.9% for anti-HBs only was found for staff that received three doses of vaccine, which is in agreement with other studies^{1 16 18}. Males, as well as, staff members over 46 years of age showed a smaller seropositive rate to the vaccine. These data agree with those of other studies, which consider that these characteristics are responsible for a failure in the immunological response to the vaccine^{1 12 17}. A lower seropositive rate to the vaccine was also observed in individuals that had little education. Of this group only 50% received the three doses of vaccine (data not shown) and in this way this fact could be related to the lower positive rate to the vaccine.

This study showed that HBV vaccination rate in Goiânia, GO has increased over time but the seroprevalence of HBV infection remains unchanged. This reinforces the necessity of taking preventive measures, including vaccination, in order to control of the virus.

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REFERENCES

- Assad S, Francis A. Over a decade of experience with a yeast recombinant hepatitis B vaccine. Vaccine 18: 57-67, 2000.

2. Azevedo MSP, Cardoso DDP, Martins RMB, Daher RR, Camarota SCT, Barbosa AJ. Rastreamento sorológico para hepatite B em profissionais de saúde na cidade de Goiânia-Goiás. *Revista da Sociedade Brasileira de Medicina Tropical* 23: 157-162, 1994.
3. Birrer RB, Birrer D, Klavins JV. Hepatocellular carcinoma and hepatitis virus. *Annals of Clinical and Laboratory Science* 33: 39-54, 2003.
4. Center For Diseases Control And Prevention. Viral Hepatitis. Exposure to blood. What health-care workers need to know. Disponível em: <<http://www.cdc.gov/ncidod/diseases/hepatitis>>. Acesso em: 05 mar, 2002.
5. Coelho HSM, Artemenko SMT, Martins CN, Carvalho DM, Valente J, Rodrigues EC, Alves LS, Martins MLM. Prevalência da infecção para o vírus da hepatite B na comunidade hospitalar. *Revista da Sociedade Brasileira de Medicina Tropical* 23: 71-76, 1990.
6. Davis GL. Update on the management of chronic hepatitis B. *Reviews in Gastroenterological Disorders* 2: 106-115, 2002.
7. Dreier J, Kröger M, Diekmann J, Götting C, Kleesiek K. Low-level viraemia of hepatitis B virus in an anti-HBc- and anti-HBs-positive blood donor. *Transfusion Medicine* 14: 97-103, 2004.
8. Fernandes JV, Braz RFS, Neto FVA, Silva MAD, Costa NFD, Ferreira AM. Prevalência de marcadores sorológicos do vírus da hepatite B em trabalhadores do serviço hospitalar. *Revista de Saúde Pública* 33: 122-128, 1999.
9. Gomes AS, Yoshida CFT, Niel C. Detection of hepatitis B virus DNA in hepatitis B surface antigen-negative serum by polimerase chain reaction: evaluation of different primer pairs and conditions. *Acta Virologica* 40: 133-38, 1996.
10. Largura MA, Pacheco M, Largura A. Ausência do HBV-DNA por PCR em Soros de Doadores de Sangue com HBsAg Negativo e Anti-HBc Positivo em Região de Alta Prevalência de Hepatite B. *Laes & Haes* 125: 106-126, 2000.
11. Lopes CLR, Martins RMB, Teles AS, Silva SA, Maggi PS, Yoshida CFTY. Perfil soroepidemiológico da infecção pelo vírus da hepatite B em profissionais das unidades de hemodiálise de Goiânia-Goiás, Brasil Central. *Revista da Sociedade Brasileira de Medicina Tropical* 34: 543-548, 2001.
12. Martínez NT, Burillo JMT, Bermúdez BP, Álvarez JBS. Factores asociados a una respuesta inadecuada a la vacunación contra la hepatitis B em personal sanitario. *Revista Espanola de Salud Publica* 72: 509-515, 1998.
13. Niel C, Moraes MTB, Gaspar AMC, Yoshida CFT, Gomes SA. Genetic diversity of hepatitis B virus strains isolated in Rio de Janeiro, Brazil. *Journal of Medical Virology* 44: 180-186, 1994.
14. Silva CO, Azevedo MSP, Soares CMA, Martins RMB, Ramos CH, Daher RR, Cardoso DDP. Seroprevalence of hepatitis B virus infection in individuals with clinical evidence of hepatitis in Goiânia, Goiás. Detection of viral DNA and determination of subtypes. *Revista do Instituto de Medicina Tropical de São Paulo* 44: 331-334, 2002.
15. Zanalda BC, Manterola AC, Lestrem MD, Frider B, Zocchi GA, Fainboim H, Clua GI, Amor E. Prevalencia del anticuerpo contra el antígeno central del virus de la hepatitis B (anti HBc) em personal hospitalario de Buenos Aires. *Boletim Sanitário Panamericano* 108: 16-24, 1990.
16. Zanetti AR. Update on hepatitis B vaccination in Italy 10 years after its implementation. *Vaccine* 19: 2380-2383, 2001.
17. Zuckerman JN. Nonresponse to hepatitis B vaccines and the kinetics of anti-HBs production. *Journal of Medical Virology* 50: 283-288, 1996.
18. Williams JL, Christensen CJ, McMahon BJ, Bulkow LR, Cagle HH, Mayers JS, Zanis CL, Parkinson AJ, Margolis HS. Evaluation of the response to a booster dose of hepatitis B vaccine in previously immunized healthcare workers. *Vaccine* 19: 4081-85, 2001.
19. Wolff M. Occupational accidents with exposure to biological fluids. Recommendations for the management of exposed workers. *Revista Medica de Chile* 125: 605-613, 1997.