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SEROPREVALENCE OF HEPATITIS B VIRUS INFECTION IN INDIVIDUALS WITH CLINICAL EVIDENCE OF HEPATITIS IN GOIÂNIA, GOIÁS. DETECTION OF VIRAL DNA AND DETERMINATION OF SUBTYPES

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SUMMARY

The presence of serological markers for hepatitis B virus (HBsAg, anti-HBc IgM and Anti-HBc total) was investigated in the serum of 1,396 individuals who had clinical suspect of hepatitis. It was observed that 50.7% of the individuals were positive and, from the total of the studied individuals, 14.5% were positive for HBsAg. From these, 8.5% were also positive for anti-HBc IgM. The analysis in relation to gender showed a higher seroprevalence index among male individuals ($p < 0.0001$). It was observed the occurrence of subtypes adw_2 (62.7%), ayw_3 (23.5%), ayw_2 (9.8%) and adw_4 (3.9%). The viral DNA was detected in 61 (33.9%) HBsAg positive samples and in one sample positive only for anti-HBc total. These results indicate an important incidence of the HBV infection in this population, and reinforce previous studies regarding this virus in the central west region of Brazil.

KEYWORDS: Hepatitis B; HBV-DNA; Subtypes.

INTRODUCTION

The hepatitis B virus (HBV) is disseminated throughout the human population and it is estimated that there are over 350 million chronic carriers of the virus, which represents approximately 5% of the world population[22]. The virus may cause in the host both asymptomatic and symptomatic infection and the disease might evolve to cirrhosis and hepatocelular carcinoma[24,33]. The HBV presents genetic variability that is demonstrated by the occurrence of subtypes and genotypes which present different distribution in relation to the geographical area[2,12,23]. Several investigations have been made regarding HBV aiming at the characterization of the positive samples for the virus, since this procedure is also useful in the comprehension of the development of the infection[21,42].

In Goiânia-Goiás, studies about HBV seroprevalence have been developed and the important spreading of the agent in this region has been demonstrated[3,4,6,7,32,39]. However, there is no data regarding this virus in population that presents clinical suspect of hepatitis. This study aims at the detection of serological markers for HBV and the viral DNA, as well as the determination of HBV subtypes in this population group.

MATERIAL AND METHODS

Studied population: From August 1995 to July 1997, 1,396 blood samples were obtained from equal number of patients with clinical evidence of hepatitis which attended the health public system of Goiânia-GO. The clinical suspect of hepatitis included at least one of the following characteristics (linked epidemiological data): low fever, jaundice, gastric intestinal symptoms (nausea and vomit) and elevations of serum aminotransferase activities. From this population, 897 were male and 499 female.

METHODOLOGY

1 - Detection of serological markers for HBV: The 1,396 blood samples were tested aiming at the detection of hepatitis B surface antigen (HBsAg), IgM antibodies to hepatitis B core antigen (anti-HBc IgM) and total antibodies to hepatitis B core antigen (anti-HBc total). For this procedure enzyme immunoassay (EIE) commercial kits were used (Hepanostika-Organon Teknika B.V., Boxtel, Netherlands). The assays were performed as described by the manufacturers.

2 - Subtyping of the HBsAg positive samples: The HBsAg positive samples were titled by reverse passive hemaglutination (Biomanguinhos/Fiocruz/Rio de Janeiro/Brazil) and the samples that presented a title equal or higher than 28 hemaglutination units were subtyped by radial immunodiffusion. The samples that presented a title between 24 and 28 units were analyzed by EIE with monoclonal antibodies. All immunobiologicals used in both assays were prepared and supplied by the Center of National Reference in Viral Hepatitis/Oswaldo Cruz Foundation/Rio de Janeiro-RJ/Brazil.

3 - DNA detection: The HBsAg positive samples were tested by

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two methodologies for detection of viral DNA: 85 were tested by molecular hybridization (Dot-blot) and 95 by polymerase chain reaction (PCR). Moreover, 28 positive samples for Anti-HBc total and negative for HBsAg were also tested by PCR. The methodology of Dot-blot was performed in agreement with NIEL *et al.* (1994)[28] with modifications. The probe was labelled with [^{32}P] dATP using the kit Megaprime DNA labeling system RPN 1604 (Amershan Biosciences, Buckinghamshire, UK).

For the PCR procedure, the DNA was extracted from serum samples and amplified according to description of NIEL *et al.* (1994)[28]. First, the samples were tested using 5 pairs of primers for the conserved areas of genome (PS1-PS2, X1-X2, C1-C2, C1-PS2 and PS1-S2). The negative samples in this first stage, were then submitted to the semi-nested PCR, using the pairs of primers PS1-PS2 and PS4-S2[15,28]. The PCR procedure utilized is able to detect 100 copies per genome (Gomes, S.A. – personal communication).

RESULTS

1 - Seroprevalence: It was observed a seroprevalence index of 50.7% (HBsAg/anti-HBc IgM; HBsAg/anti-HBc total; anti-HBc total). From the total of 1,396 individuals, 14.5% were positive for HBsAg and 8.5% were also positive to anti-HBc IgM (Table 1). Moreover, from the 897 male individuals, 60.1% were positive in relation to 33.9% of the female individuals ($p < 0.0001$) (Table 2).

Table 1
Distribution of hepatitis B virus positive serum samples, obtained from individuals with clinical evidence of hepatitis, in relation to the serological markers

	N	%*
HBsAg/ anti-HBc IgM	119	8.5
HBsAg/ anti-HBc total	83	6.0
Anti-HBc total	506	36.2
Total	708	50.7

*Index calculated in relation to the 1,396 blood samples analyzed; N- number of positive samples.

Table 2
Distribution of hepatitis B virus positive samples obtained from individuals with clinical evidence of hepatitis, in relation to the gender

	Positives/Total	%
Male*	539/897	60.1
Female	169/499	33.9
Total	708/1396	50.7

*$X^2 = 88.2$ $p < 0.0001$

2 - Subtyping: From the subtyped samples, 32 (62.7%) were adw_2, 12 (23.5%) ayw_3 and the remaining samples were ayw_2 (9.8%) and adw_4 (3.9%). The analysis in relation to the year shows that the subtype adw_2 occurred throughout the years of study, while the subtypes ayw_3 and adw_4 occurred only during the year of 1996 and the ayw_2 in 1995 and in 1996 (Table 3).

Table 3
Distribution of HBsAg subtypes from 51 hepatitis B virus positive samples obtained from individuals of Goiânia-Goiás in relation to the year of collection

Year	adw_2	ayw_3	ayw_2	adw_4	Total
1995	10	-	01	-	11
1996	11	12	04	02	29
1997	11	-	-	-	11
Total	32 (62.7%)	12 (23.5%)	05 (9.8%)	02 (3.9%)	51 (100.0%)

3 - Detection of viral DNA: Positivity indexes of 35.3% and 32.6% were observed for viral DNA using the methodologies of Dot-blot and PCR respectively (Table 4). From the 28 negative serum samples for HBsAg and positive for anti-HBc total, one was positive for viral DNA.

Table 4
Detection of HBV-DNA by Dot-blot and PCR methodologies, in relation to the serological markers, in individuals with clinical evidence of hepatitis from Goiânia-Goiás

	Dot-blot		PCR	
	N	(%)	N	(%)
HBsAg/anti-HBc IgM	19/42	(45.2)	19/64	(29.7)
HBsAg/anti-HBc total	11/43	(25.6)	12/31	(38.7)
Total	30/85	(35.3)	31/95	(32.6)

N - number of positive samples/ number of tested samples

It was also observed that the pair C1-C2 presented the highest index (21.6%) of detection of the viral DNA followed by X1-X2 (12.6%) and PS1-PS2 (11.6%).

DISCUSSION

Although there are many epidemiological surveys regarding HBV seroprevalence reported in literature, the occurrence of HBV in clinical cases of hepatitis has been poorly documented. In this way, few studies have been carried out with population similar to this study. In a study by CASEY *et al*. (1996)[9] performed in Peru, a seroprevalence index of 100% was observed. In the present study, a global index of 50.7% of HBV seropositivity was detected. Comparing this result with data from the literature, concerning other population, it was observed that this index is higher than several other indexes detected in different parts of the world[1,8,13,20], including Goiânia-Goiás[3,6,32].

The analysis of the distribution of the serological markers for HBV shows that 14.5% of the individuals were positive for HBsAg and 8.5% were also positive for anti-HBc IgM, which indicates not only the condition of carriers of the virus, but also acute infection. These indexes were higher when compared to studies accomplished in different areas of the world with populations different from this study[20,37,40], the same occurring in Brazil[30,36], including Goiânia-Goiás[4,34,39]. Also, it was

observed that other studies accomplished with similar population presented higher HBsAg indexes of detection, such as 59.3% in patients with hepatocarcinoma in Nigeria[31], 60.0% in patients with acute hepatitis in Somalia[29] and 88.0% in military recruits from Peru[9].

The data showed higher detection of HBV (60.1%) among male individuals, which was also demonstrated by other studies[1,26].

In this study it was observed a predominance of the subtype adw_2 followed by ayw_3, which is in agreement with other studies[14,39], and supports previous results that have demonstrated a higher prevalence of these subtypes during the last decade in the region of Goiânia, Goiás, Brazil[14].

Indexes of viral DNA detection of 35.3% and 32.6% were observed by Dot-blot and PCR, respectively. These results are in agreement with the studies accomplished by TEDESCHI *et al*. (1989)[38] and CHIARAMONTE *et al*. (1991)[11] although they differ from other studies in which it has been observed higher indexes of detection for HBV-DNA by PCR[2,16,27] and Dot-blot[26].

A higher detection of the viral DNA was observed when the C1-C2 primer pair was used, and this fact is in agreement with previous study[15]. Although some studies have been observed the occurrence of mutations in the genomic area Pre-C/C[21,43], it is assumed that it is a conserved genomic area[17] and, in this way, the present study confirmed the effectiveness of this pair of primers for HBV-DNA detection.

From the 28 anti-HBc total positive samples tested by PCR, one was positive. Some reports have shown the detection of the HBV-DNA by PCR in individuals positive for anti-HBc total/ anti-HBs, and even in individuals without any serological marker for the infection[15,35,41] and some explanations have been proposed to elucidate this condition[5,18,19,43]. In any case, this fact supports the importance of screening by anti-HBc at the blood banks from all areas of the world and point out the requirement of improving the screening methods used for viral detection[10,15,25,35].

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RESUMO

Soroprevalência da infecção pelo vírus da hepatite B em indivíduos com evidência clínica de hepatite em Goiânia, Goiás. Detecção do DNA viral e determinação dos subtipos

Investigou-se a presença dos marcadores sorológicos AgHBs, anti-HBc IgM e anti-HBc total no soro de 1.396 indivíduos com suspeita clínica de hepatite. Observou-se uma soroprevalência para a infecção pelo VHB de 50,7%, sendo que do total dos 1.396 indivíduos, 14,5% eram positivos para AgHBs e 8,5% eram também positivos para anti-HBc IgM, tendo ainda sido observado que a soroprevalência foi maior em indivíduos do sexo masculino ($p < 0,0001$). Por subtipagem foram identificados os seguintes subtipos: adw_2 (62,7%), ayw_3 (23,5%), ayw_2 (9,8%) e adw_4 (3,9%). O DNA viral foi detectado em 61 (33,9%) amostras positivas para o AgHBs e em uma amostra positiva somente para anti-HBc total. Estes resultados indicam importante índice de ocorrência da infecção pelo VHB nesta população e reforça dados de estudos anteriores a respeito da importante circulação do vírus na região Centro-Oeste do Brasil.

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