The Hepatitis Delta Virus, Pages 105–113

ASSESSMENT OF DELTA VIRUS INFECTION IN VENEZUELAN HIGH-RISK POPULATION FOR HEPATITIS B VIRUS

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ABSTRACT

HDV infectivity particularly related to sexual activity has been difficult to establish. We investigated the prevalence of HDV in a high risk urban male population currently evaluated for HIV infection. Fourth-eight homosexual or bisexual men (96% positive for HIV) being routinely followed in the outpatient clinic, 40 sera obtained randomly from male homosexuals and 24 HBsAg carriers were examined by ELISA and Western Blot. HDV RNA was assessed by slot-blot after hybridization with cDNA probe from a recombinant plasmid (pS-1). [None of the 48 male subjects or from a recombinant plasmid (pS-1).] None of the 48 male subjects or from the randomly selected homosexuals tested positive for anti-HDV. HDV RNA searched in a selected group of sera from either high risk population or from HBsAg carriers proved also to be negative. We suggest that factors other than HBV chronic bearing and/or sexual promiscuity should be associated with HDV spread.

INTRODUCTION

Hepatitis Delta Virus (HDV) infection superimposed to endemic areas of Hepatitis B Virus (HBV) disease has been widely reported (Howard C, 1986). In Venezuela, outbreaks of HDV seemed to occur since 1975 and both superinfection and coinfection in HBV [coinfection] in HBV chronic carriers have been circumscribed to Amerindians communities (Popper et al, 1983; Hadler et al 1984; Machado et al, 1988). Since the initial reports of HDV infection, polytransfused patients as well as intravenous drug addicts have been identified as high-risk populations to contract HDV infection (Rizzetto et al, 1980; Hansson et al, 1982; Carredda et al, 1984). Worldwide, the spread of HDV share epidemiological characteristics with HBV and Human Immunodeficiency Virus (HIV) infections, both classified as blood-transmitted diseases (Goedert et al, 1985). Other means of HDV infectivity, particularly related to sexual activity has been difficult to establish and remains a controversial issue (Rizzetto et al, 1988). Within this context, the prevalence of HDV infection seems not to be influenced by HBV penetration. Furthermore, in some HBV endemic areas significant spread of HDV has not been encountered (Rizzetto et al, 1988). For instance, Venezuela is classified as an intermediate region for the prevalence of HBsAg chronic carriers (Machado et al, 1985; Machado I, 1986), however, very few cases of HDV infection have been notified in Venezuelan urban areas. We herein report seroepidemiological studies of HDV prevalence assessed in a high risk urban male population being evaluated for the presence of HIV infection. The survey also included a group of well characterized Venezuelan HBsAg chronic carriers.

MATERIAL AND METHODS

URBAN MALE HIGH-RISK POPULATION

Forty-eight individuals, age range between 19 and 41 years (mean, 29 years), were selected at random among an average of 120 patients attending monthly our Immunodiagnostic laboratory for HIV screening. The total group are part of an ongoing clinical-epidemiological protocol already established within our patient care department (Bianco N, 1986; Perez et al, 1989). Twenty five were homosexuals and 23 bisexuals with no history of intravenous drug addiction. The group was screened for HIV and HBV serological markers. HDV antibodies were

investigated independently of the HBV antibodies were investigated independently of the HBV serological status. In addition, 40 serum samples randomly obtained from male homosexuals not included in the clinical-epidemiological protocol were also studied.

HBsAg CHRONIC CARRIERS POPULATION

Twenty-four HBsAg chronic carriers, mean age: 35 years (20 male and 4 female) were also tested for the presence of HDV serological markers. The group was divided into 2 subgroups: 9 symptomatic patients including 2 chronic persistent hepatitis, 5 chronic active hepatitis, 2 liver cirrhosis, all diagnosed by liver histopathology and 15 asymptomatic patients without indication of liver biopsy.

HIV

Anti-HIV antibodies were detected by enzyme-immuno assays (ELISA) (Vironostika, Organon-Teknika, Turnhout, Belgium) and confirmed by two commercial western-blot assays (Dupont Company, Wilmington USA; Organon-Teknika, Durham, USA). Both techniques are routinely used in our laboratory (Perez et al, 1989).

HBV

ELISA was also used for the detection of HBsAg, anti-HBs and total anticore (IgM + IgG) (Hepanostika; Organon-Teknika, Organon-Tenika Turnhout, Belgium). HBsAg chronic carriers were also studied for the presence of HBeAg or anti-HBe using a microelisa technique (Hepanostika, Organon-Teknika, Belgium).

HDV

The presence of anti-HDV antibodies (total anti-HD) was determined by a microelisa system developed by Organon-Teknika, The Netherlands (Hepanostika, Turnhout, Belgium). Validity of this method has been described elsewhere in detail (Dubois et al, 1988).

HYBRIDIZATION ASSAY

HDV RNA was detected in 6 selected serum from the homosexual population: three HBsAg positive samples, one anti-core positive sample and two HIV positive serum with negative HBV markers. Besides, 11 serum form the HBsAg chronic carrier group was analyzed for the presence of HDV RNA. Detection of Delta virus RNA in serum samples was

carried out by slot-blot. The recombinant plasmid p-1 containing 520 base pairs (bp-Delta virus cDNA sequence, cloned in the PstI site of pBR 322, was kindly provided by Dr. Colin Howard (London School of Hygiene and Tropical Medicine). The cDNA probe was labeled by the oligonucleotide random priming method with digoxigenin DUTP according to instructions of the manufacturer (Boehringer-Mannheim, USA). Serum samples were digested with 2% SDS and 1 mg/ml of proteinase K at 37C for 1 hr. Following extraction (twice) with phenol/chloroform (1:1) and once with chloroform: isoamylalcohol (24:1), the nucleic acids were precipitated with 2 volumes of ethanol. The precipitate was dried and resuspended in sterile double distilled water. After denaturation with Glyoxal 1M and Dimethylsulfoxide 50%, the samples were applied to a slot-blot apparatus under vacuum suction (Bethesda Research Laboratories, Rockville, MD). Hybridization was performed at 42C in 50% formamide with digoxigenin labeled probe. Highly stringent conditions were used for the washing steps including 0.1 SSC, 0,1% SDS at 65C. Finally, filters were incubated with alkaline phosphate-labeled anti-digoxigenin antibody and developed with Nitroblue Tetrazolium Salt (NBT) and 5-bromo-4-chloro-3-indonyl phosphate (X-phosphate) as substrate.

RESULTS

HIV

Forty-eight subjects (96%) had positive anti-HIV antibodies confirmed by western blot (Table 1).

HBV

Twenty-three individuals showed one or more HBV positive markers: 4 positive HBsAg and 19 positive total anti-core with or without anti-HBs. When the presence of HBV markers was correlated with number of sexual partners we found that 19 patients had 5 or more sexual partners per year while 29 reported less than 5 partners. As described in Table 1, 100% from the first group (19 subjects) showed at least one positive HBV marker while only 14% (4 individuals) of the later group demonstrated any of the HBV serological markers ($p<0.001$, Chi^2 with Yate's correction). No difference was noted related to the homosexual or bisexual condition.

HDV

None of the 48 male subjects tested showed positivity

for total anti-HD. Similarly, HDV RNA was negative in the 6 serum tested. Furthermore, the 40 male homosexuals randomly searched for anti-HD antibodies also showed negative results.

HBsAg CHRONIC CARRIER POPULATION

Only one HBsAg positive patient demonstrated a positive anti-HD with consistently positive results during his follow-up. His liver histology demonstrated a chronic active liver disease and he has remained HDV RNA negative and anti-HBe positive through one year of follow-up. It is important to notice that this patient actually living in the capital city had lived for many years in the South of Venezuela, in a well known endemic HBV and HDV area. The remaining 23 HBsAg chronic carriers were negative for anti-HD antibodies. In this later group, eleven selected serum investigated for HDV RNA proved also to be negative (Table 2).

DISCUSSION

Outbreaks of HDV infection have been well described in some Latin American countries (Hadler et al, 1984, Da Fonseca et al, 1985, Machado et al, 1988). Worldwide however, there is a high variability in the prevalence of HDV and sporadic cases of HDV infection might represent modes of HDV transmission still unknown. In this sense, it has been suggested that the penetration of HDV might be influenced by other unrecognized factors not necessarily determined by the proportion of HBV infection (Rizzetto et al, 1988).

Venezuela is classified as a country with intermediate prevalence of HBV chronic (Machado et al, 1985; Hadler et al, 1987). Furthermore, hyperendemic geographical areas for HDV infection, particularly circumscribed to the amerindian communities, located at the Northwest and South of Venezuela have been described (Hadler et al, 1984; Machado et al, 1988). On the other hand, the HBV infection in urban zones seems to prevail in the poorest areas of the cities although an increasing proportion of HBV infection has recently been detected nationwide, not always related to low social-economical factors (Machado I, 1986; Fernandez et al, 1988). Within this later group the male homosexual population with increased rate of HBV contacts has been included (Machado et al, 1988). In the present study, we

found that male individuals at high-risk to contract HBV and/or HIV infections proved to be negative for HDV markers. Opposite to intravenous drug addicts whose HDV high risk characteristic has consistently been reported, (Caredda et al, 1984, De Cock et al, 1986, Rizzetto et al, 1988) the rate of HDV infection in male homosexuals remains controversial tending to vary from one country to another. For instance, in a recent HDV survey in homosexual men in the United States, HDV was considered an infrequent cause of viral hepatitis (Weisfuse et al, 1989) while in French male HBsAg positive homosexuals it was concluded that this population is at particular risk of infection as suggested by a wide range of serological profiles (Pol et al, 1989). Furthermore, within the country prevalence of HDV in the homosexual population may differ. Thus, Delta infection was identified in homosexual men from the West Coast of the United States (Solomon et al, 1988). The possibility that HDV infection could behave as a sexually transmitted disease has arisen from those studies showing HDV penetration in high-risk male individuals (Pol et al, 1989, Solomon et al, 1988). However, in our study none of the high risk homosexual subjects showed circulating HDV markers while 96% and 48% demonstrated HIV and HBV serological markers, respectively. Furthermore, the HBV prevalence also correlated with the number of annual sexual partners which reinforces sexual activity as a major risk factor, either in homosexuals or bisexuals. Therefore, the absence of positive HDV individuals in our study tends to indicate that factors other than HBV chronic carriage and/or sexual promiscuity may be related to the sporadic appearance of HDV cases.

In conclusion, in Venezuela, the known risk factors of HDV infection should be consistently sought independently of the sexual orientation of the patient. Emphasis should be placed on past contact with Amerindian communities. Furthermore, additional insight may be obtained if HDV epidemiological data is accumulated from other countries with intermediate prevalence for HBV infection.

TABLE 1

HIV, HBV AND HDV SEROLOGICAL MARKERS IN HIGH-RISK VENEZUELAN MALE POPULATION

*No of Subjects	No of Sexual Partners Annually	No. of positive serum Anti-HIV	HBsAg	Anti-core/Anti-HD
19	> 5	19	3	16	0
29	< 5	27	1	3	0

* 6 Selected Serum; HDV RNA Negative

TABLE 2

HBV AND HDV SEROLOGICAL MARKERS IN HBsAg VENEZUELAN CHRONIC CARRIERS

*No of Patients	** ALT IU/L	HBeAg +ve	HBeAg -ve	Anti-HBe +ve	Anti-HBe -ve	Anti-HD +ve	Anti-HD -ve	HDV RNA +ve	HDV RNA -ve
9	168	5	4	4	5	1	8	0	9
15	70	8	7	5	10	0	15	0	15

All were HBsAg +ve; Total Anti-core +ve, Anti-HBs -ve

* Serum samples tested for HBV DNA: 8 +ve, 3 -ve (Slot-blot with non-radioactive labeled probe vs. radioactive probe; manuscript in preparation).

** Mean ALT value.

ACKNOWLEDGEMENTS

This work was done with grants from Fundacion Pollar and Immunobilogia Asociacion Civil, Caracas.

Thanks are due to Mrs. Merly Marquz for her technical assistance, to Dr. Freya Capriles for providing us with serum samples, and to Miss Hilda Rodriquez for typing the manuscript.

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