HBsAg subtype distribution among different populations of HBsAg carriers in Spain

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Abstract. Data concerning the HBsAg subtype distribution in Spain are out-of-date and confined to a restricted geographical area. Furthermore, the complex distribution observed in the countries surrounding Spain prevents any prediction. To obtain further data on HBsAg subtype distribution among Spanish HBsAg carriers, subtyping analysis (d and y determinants) was performed in 670 samples from subjects belonging to various epidemiological risk groups and coming from different geographical areas of the country. Similar frequencies were found for both mutually exclusive d/y subtype determinants among non-risk, normal HBsAg carriers from almost all geographical areas studied. In contrast, the ay subtype was clearly predominant (79–87%) among intravenous drug users, irrespective of their geographical origin. Thirteen different institutions for mentally retarded patients behaved as closed communities for HBV circulation, showing independent subtype distributions. Thus, no significant geographical variations were found for HBsAg subtype distribution in Spain. The prevalence of each particular subtype is mainly dependent on the epidemiological characteristics of the carriers studied. Subtype distribution was independent of the presence of HBeAg or HDV infection serum markers when homogeneous groups were considered separately. Atypical HBsAg phenotypes, either with coexistence or absence of both subtype determinants, were found in some cases.

Key words: HBsAg subtypes, HBV variants, Hepatitis B virus

Introduction

The hepatitis B surface antigen (HBsAg) is the major protein of the hepatitis B virus (HBV) envelope. It has a common antigenic determinant, called a [16], and one member of each pair of mutually exclusive determinants, d or y [14], and w or r [1]. Consequently, there are four main antigenic subtypes of HBsAg (adw, ayw, adr and ayr). Additional sub-specificities assigned to the w determinant have been described, leading to a nine-member classification, namely adw2, adw4, ayw1, ayw2, ayw3, ayw4, adwr+, adwr-, ayr [3, 5]. Most of the antibody elicited against the HBsAg (anti-HBs) during the normal immune response are of the anti-a specificity, leading to cross-protection between different subtypes [19]. Vaccine escape variants due to mutations in the common determinant coding region have been described [2, 40]. Unusual coexistence of mutually exclusive subtype determinants as a result of phenotypic mixing has also been found [23, 25, 35, 38], as well as rare variants apparently lacking subtype antigenic expression [24, 35].

Each HBsAg subtype has a particular geographical distribution [4, 17, 32]. Territorial variations of this distribution within a country [6, 12, 31, 39] or among different populations living in the same geographical area [34, 37] have been reported, however. Furthermore, temporal changes in the patterns of subtype distribution have also been found [11, 27, 36].

Although HBsAg subtypes are generally considered to be clinically and pathologically irrelevant [9, 15, 30], several associations between subtypes and hepatic injury [10, 41] or active replication serum markers [13, 20] have been described.

Data concerning HBsAg subtype distribution in Spain are out-of-date and restricted to Barcelona [26, 27]. The complex distribution in the surrounding countries [4] prevents any prediction about subtype distribution in the whole country. In the present report, we show results of HBsAg subtype distribution among several risk populations for HBV infection from different areas of Spain. Correlations between subtypes and the presence of Hepatitis B e antigen (HBeAg) and Hepatitis Delta Virus (HDV) infection markers are also studied. Some cases of unusual subtypes are described.
Materials and methods

Patients and samples. The study was based on 611 serum samples taken between 1988 and 1992 from Spanish HBsAg carriers, grouped in two categories. Patients from group 1 included 545 HBsAg carriers from nine different locations: 148 from Madrid (Centro Nacional de Microbiologia, Virologia e Inmunologia Sanitarias), 141 from Asturias (Laboratorio de Salud Publica del Principado de Asturias), 58 from Zaragoza (Hospital Miguel Servet), 75 from Málaga (Hospital Carlos Haya and Banco de Sangre de Málaga), 25 from Barcelona (Hospital de la Santa Creu i Sant Pau), 27 from Pamplona (Banco de Sangre de Navarra), 47 from Sevilla (Centro Regional de Transfusion Sanguinea de Sevilla) and 24 from Valladolid (Hospital Universitario de Valladolid). Two hundred and seventy-one patients lacked any known HBV infection risk factor (NRF) (187 blood donors from Oviedo, Zaragoza, Banco de Sangre de Málaga, Navarra and Sevilla; 84 patients from Madrid and Valladolid); 181 were intravenous drug users (IDU) from Madrid, Asturias, Zaragoza, Hospital Carlos Haya of Málaga, and Barcelona; 93 from Madrid and Asturias had other risk factors (ORF). Cases were either consequently recruited or random selected from collections of samples, with exception of those from Navarra. They were follow-up samples from volunteer blood donors from the same rural area who were detected as HBsAg carriers more than fifteen years ago (Dr. J.L. Martinez, Banco de Sangre de Navarra, personal communication). Age data were only available from Madrid (mean age 21.7 years for NRF, 24.8 for IDU and 27.7 for ORF).

Group 2 included 66 samples from patients in closed or semi-closed institutions. Fifty-five samples were from patients in institutions for the mentally retarded, the remaining eleven being inmates from a single prison.

The specificity of subtyping methods described below was tested with an HBsAg subtype reference panel [3] from the Centre Nationale de Transfusion Sanguine, Paris (kindly supplied by Dr. A.M. Couroucé) and the National Institute of Allergy and Infectious Diseases, USA (kindly supplied by Dr. D. Peterson). This panel contains samples of the following subtypes: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+, adrq- and a ‘mixed’ subtype adyw.

Methods. Monoclonal antibodies (Mab) used in HBsAg subtyping were H35C (a-specific), H95C (d-specificity) and H10C (y-specificity), from Abbott Laboratories, Chicago, USA [29] (kindly supplied by Drs. D.R. Decker and S.G. Devare).

Three enzyme immunoassays (EIA) were used. The first was an indirect assay [7], in which the HBsAg contained in samples was captured into microtiter wells coated with guinea pig anti-HBs. Subtype epitopes were then tested with their corresponding Mab and peroxidase-conjugated goat anti-mouse antiserum.

The second method (neutralization EIA) was a modification of the Auszyme Monoclonal EIA test (Abbott Labs) for HBsAg detection [8]. Serum samples were previously incubated with H35C and H95C Mab, as well as with normal calf serum as base control, and free HBsAg determined by the normal Auszyme protocol. Results were given as percent of neutralization in reference to the control value.

The third method was an inhibition EIA assay. Briefly, 100 μl aliquots of each sample were mixed with 10 μl of each diluted Mab. A reference absorbance control with human negative serum was included for each Mab. After incubation for one hour at 37 °C, mixtures were added to separate microtiter wells coated with HBsAg (Biokit Labs., Barcelona, Spain). After overnight incubation at room temperature, wells were washed and solid phase-bound Mab determined by adding 100 μl of peroxidase-conjugated, goat anti-mouse IgG. After incubation for one hour at 37 °C, colour was developed as in the indirect assay. The ability of each sample to prevent MAb binding to the solid phase was expressed as the percentage of reduction compared to the value of the Mab control. Values greater than 40 were considered positive.

The sensitivity of these assays was estimated using an HBsAg standard panel. The sensitivity of the indirect assay, expressed as ng/ml, was 3 (ad) and 1.8 (ay) for the a determinant, 8 for the d determinant and 7 for the y determinant. Sensitivity of the neutralization assay was given by the sensitivity of auszyme test, estimated at approximately 0.1 ng/ml. The inhibition assay was estimated to be 10-fold less sensitive than the indirect assay.

Statistical analysis. Statistical significance of the results obtained was tested by the chi-square test with Yate’s correction.

Results

HBsAg subtype reference panel. Results obtained using the HBsAg subtype panel are shown in Table 1. In the indirect assay all samples were recognized by the a-specific Mab, as were the ay samples by the H10C Mab, and the ad samples by the H95C Mab. However, the adw4 and adr sera showed crossreactivity with the H10C Mab, with ratios lower than 5.0. This crossreactivity was diminished in the inhibition assay, so that only the adr+ sample remained positive for both subtype determinants. The adyw sample was also reactive for both subtype-specific Mab, but it showed high values for both determinants in the inhibition EIA. All ad panel members could be