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Subtypes of Hepatitis B Surface Antigen in Turkey

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Hepatitis B virus (HBV) is an etiologic agent of acute and chronic diseases throughout the world. Turkey is an area of intermediate endemicity of viral hepatitis in the world, and hepatitis B (HB) remains one of the most important diseases to be controlled in this country. Since 1992, hepatitis vaccination among young children has been included in the Expanded Program of Immunization (EPI) by using recombinant HB vaccines containing subtype antigens *adw* and *adr*. A seroepidemiological study on HB surveillance in Turkey has been in progress for a long time (1). However, the distribution of subtypes of hepatitis B surface antigen (HBsAg) in this country has not yet been elucidated.

The serological heterogeneity of HBsAg has been established. HBsAg has a common determinant termed *a*, mutually exclusive subtype determinants called *d* and *y*, and another mutually exclusive set of *w* and *r*, thus demonstrat-

ing four major subtypes of HBsAg, *adw*, *adr*, *ayw* and *ayr*. Additional subtypes have also been characterized, so that HBV strains are now classified into nine subtypes, *ayw*₁, *ayw*₂, *ayw*₃, *ayw*₄, *adw*₂, *adw*₄, *ayr*, *adrq*⁻ and *adrq*⁺ (2). More recently, a genetic classification of HBV was proposed, including six groups designated from A to F (3). The correlation between subtypes and genotypes has also been studied (3,4)

It has been well documented that subtypes and genotypes of HBV have distinct geographic distributions, corresponding to the country of origin of chronic carriers; thus, they are valuable in tracing the source of infection and estimating the routes of different subtypes of the disease (2-4). The purpose of the study was to provide actual information on HBsAg subtypes in Turkey, previously inferred from studies conducted for other populations in the Mediterranean region.

A total of 129 HBsAg positive sera collected from four provinces of Turkey were studied. Of these, 37 sera were from the study population in the three regions of Turkey surveyed under the project of Epidemiological Surveillance for Vaccine Preventable Diseases conducted by the Ministry of Health (5). The project collected serum samples during

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the year 2000-2001 according to a program design of community-based seroepidemiology. Three provinces of different geographic regions with various socio-economic levels were selected for the survey: Antalya, a touristic area located in the south-west of the country, Samsun in the Black Sea region in the north-west, one of the developed provinces, and Diyarbakir in the east Anatolian region, of low socio-economic level. The survey groups consisted of a healthy population of all ages with approximately equal ratios of male and female, and urban and rural areas from each region. A total of 1,879 serum samples from the three provinces in the surveillance program were tested with enzyme immunoassay for the presence of HBsAg (Murex HBsAg Version 3, Abbott Laboratories, North Chicago, Ill., USA; manufacturer: Murex Biotech Ltd., Dartford, UK). Among them 55 were positive for HBsAg and 37 were analyzed for subtypes. Among the three provinces included in the surveillance program, HBV prevalence was higher in Diyarbakir than in the other two provinces; the HBsAg positive rate for Diyarbakir was 5.2% (32/618) compared with 1.8% for both Antalya (12/649) and Samsun (11/612).

The other 92 HBsAg positive sera included for subtyping were collected from persons who visited a clinical laboratory (AIDS and Hepatitis laboratory, Refik Saydam National Hygiene Center Presidency) in Ankara, the central part of Turkey, for diagnostic purpose during the year 2001-2003.

Sex and age distributions of the total specimens studied are shown in Table 1. Subjects from the surveillance program were distributed almost equally between 2 and 72 years of age (mean 37.6 years), while those from the clinical laboratory, although ranging from 12 to 68 years of age (mean 27.2 years) were concentrated (more than 70%) in the range from 20 to 30 years. The proportions of males were 64.9% (24/37) for surveillance subjects and 67.0% (59/88) for those from the clinical laboratory.

Serum samples positive for HBsAg, both from the surveillance program and from the clinical laboratory, were subjected to subtyping using a commercial kit (HBsAg Subtype EIA, Special Immune Research, Tokyo, Japan) (6). Distribution of HBsAg subtypes in four provinces in Turkey is shown in Table 1. Of 129 serum samples positive for HBsAg, 113 (87.6%) were determined to be a subtype of *ayw*. The subtype *adw* was found in two sera. Among the other 14 sera, 5 were not clear at the position of *d/y* and 9 at *w/r*, but none of the sera was found to have *ayr* or *adr*. The surveillance samples and those from clinical laboratory were quite similar in their distributions of subtypes: the *ayw* subtype constituted 86.5% (32/37) and 88.0% (81/92), respectively, with only two samples of *adw* subtype, a 60 year-old female in Diyarbakir (a surveillance sample) and an 18 year-old male in Ankara (a clinical laboratory sample). The results showed that the great majority of HBV in Turkey is of the subtype *ayw*, regardless of the different geographical locations and HBV endemicities of the three provinces included in the epidemiological surveillance. The same result was obtained with samples from the clinical laboratory in Ankara, which attracts residents from various other regions of the country.

The result of our study is consistent with a report of other studies, which describes *ayw* as the most widespread subtype in the Near East and Mediterranean countries (2). It has also been reported that *ayw* is not a simple subtype but is split into four different categories, *ayw*₁, *ayw*₂, *ayw*₃ and *ayw*₄, and that this additional subtyping could differentiate more precisely the geographical distribution of HBV; *ayw*₁ has been

Table 1. Distribution of HBsAg subtypes in four provinces in Turkey

		<i>n</i>	Subtype			
			<i>ayw</i>	<i>adw</i>	<i>a-x</i> ^{1)-w}	<i>ay-x</i> ¹⁾
Total		129	113	2	5	9
Surveillance	Samsun	7	5	0	1	1
	Antalya	8	7	0	1	0
	Diyarbakir	22	20	1	0	1
	Subtotal	37	32	1	2	2
Clinical laboratory	Ankara	92	81	1	3	7
Sex	Male	83	71	1	4	7
	Female	42	38	1	1	2
	UK ²⁾	4	4	0	0	0
Age (years)	<10	5	5	0	0	0
	10s	6	4	1	0	1
	20s	27	24	0	1	2
	30s	38	36	0	0	2
	40s	16	14	0	1	1
	50s	12	11	0	1	0
	≥ 60	9	6	1	0	2
	UK ²⁾	16	13	0	2	1

¹⁾: Determinant was not identified.

²⁾: Unknown.

found only in Vietnam, *ayw*₂ is the predominant subtype in the Mediterranean, *ayw*₃ is prevalent in Greece and Yugoslavia along with *ayw*₂, and *ayw*₄ is mainly found in West Africa and also Central Africa along with *ayw*₂ (2). More recently, genotype analysis has been introduced, and it has been revealed that among six genotypes designated from A to F, the group D is the predominant genotype in the Mediterranean area, the Near and Middle East and in south Asia (3). When analysed based on subtype and genotype classification, the group D includes *ayw*₂, *ayw*₃ and *ayw*₄ strains (4). In addition, studies recently reported from Turkey have shown that the group D was the only detected genotype from acute or chronic HB patients in this country (7-9). These reports suggest that the subdetermination of *ayw* in combination with genotyping will be useful in providing more precise epidemiological features of the distribution of HBV in this country.

In our present study, we examined serum samples from four geographically different regions in Turkey, collected for a community-based epidemiological survey of healthy populations, along with those from a clinical laboratory. We concluded that *ayw* is the predominant subtype of HBsAg in Turkey, and can be considered to be widely spread throughout the country.

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