Seroepidemiology of hepatitis B, C and delta viruses in Tunisia

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Abstract
Serum samples from 33,363 healthy people in Tunisia have been tested for serological markers of hepatitis B, C and delta viruses (HBV, HCV and HDV). Hepatitis B surface antigen (HBsAg) was detected in 6.5% of sera. The overall seroprevalence of HBV was 37.5%. Vertical and perinatal transmission of HBV in the first 3 months of life occurred in only 0.4% of 177 mother and child pairs. HBV seroprevalence was 10.7% in infants under 2 years old and increased with age, rapidly till 27 years of age and then more slowly in adulthood, reaching 54% for people aged over 40 years. HBsAg seropositivity varied throughout the country, ranging from 3% to 13% with higher prevalences in the south and central-west regions. Overall seroprevalences for HDV and HCV were 17.7% and 4.4%, respectively. HDV superinfection occurred later than HBV and increased with age in parallel with HBV. Overall, HCV and HBV infections had different geographical distributions throughout the country. The study confirmed the high prevalence of HBV infection in Tunisia; it occurs mainly in children and teenagers, and vertical and perinatal transmission of HBV does not appear to be significant. HDV superinfection is quite common in Tunisia and occurs in almost 44% of individuals infected with HBV. In contrast, seroprevalence of HCV in the Tunisian general population was low (0.4%). These results indicate differences in the distribution of the viruses and/or different routes of transmission.

Keywords: hepatitis B virus, hepatitis C virus, hepatitis delta virus, seroepidemiology, Tunisia

Introduction
Hepatitis B is a public health problem of world-wide importance. The incidence and prevalence of the disease is variable and may differ widely depending on the rate of chronic infection with hepatitis B virus (HBV), the proportion of people with actively replicating virus, and the predominant routes of transmission in each region. Thus, in south-east Asia, vertical transmission has been shown to be the major mechanism which accounts for the high endemicity of HBV, mainly due to active viral replication in mothers (STEVENS et al., 1975; BEASLEY et al., 1983; WONG et al., 1984). In sub-saharan Africa, a region also highly endemic for HBV, the infection is acquired during the postnatal period and in early infancy and horizontal spread among siblings appears to be the most important mode of transmission, although little is known about the way in which this transmission takes place (PRINCE et al., 1981; WHITTLE et al., 1983). In North Africa and the Middle East, HBV carrier rates ranging from 4 to 10% have been reported (BAHRI et al., 1988; HOURISSA et al., 1988; AL-FALIH et al., 1992). According to these regions, these prevalences have been classified as intermediate endemicity for HBV.

Similarly to HBV, hepatitis C virus (HCV) and hepatitis delta virus (HDV) infections show wide geographical heterogeneity. Little is known about their prevalences in North Africa. Although the 3 viruses share similar modes of transmission, their distribution worldwide may differ strikingly. Thus, HDV is highly endemic in some regions like southern Italy, intertropical Africa, and Saudi Arabia (NORDENFELT et al., 1983; CENAC et al., 1986; POVEY et al., 1988; SHOBOKSHI & SEREBOUR, 1991), but, curiously, it occurs infrequently in south-eastern Asia (LIU et al., 1987; WANG et al., 1987). In most countries, rates of HCV seropositivity in the healthy population ranged from 0.2 to 1.5% (HESS et al., 1988; JANOT et al., 1989; KAMITSUKASA et al., 1989; KUHNL et al., 1989; KUO et al., 1989; SHIRSHIA et al., 1989; CHEN et al., 1990; KEOH et al., 1995; CONTRERAS et al., 1991; FROMMEL et al., 1993). Factors accounting for the geographical variability in viral distribution are largely unknown and may involve differences in virulence and pathogenicity of local strains or differences in transmission mechanisms or in host susceptibility.

In this paper, we report the seroprevalences of HBV, HCV and HDV infections in Tunisia, a North African country which has historically undergone alternating influences related to migrating populations from the north, the east and the south.

Subjects and Methods
Subjects
Thirty-three thousand, three hundred and sixty-three healthy people were included in our study, consisting of 3 groups. Group 1 was composed of 31,624 young male military recruits, aged 20–25 years, originating from all parts of Tunisia. This group was studied to assess the geographical distribution of hepatitis B surface antigen (HBsAg) carriers and HCV infection. Sera were collected between January 1987 and April 1994 and all were screened for HBsAg. Those collected after November 1993 were also screened for HCV (n=9782). Group 2 included 785 randomly selected healthy individuals (402 males and 383 females), aged from 6 months to 60 years: 167 individuals aged 6 months to 10 years, 268 aged 11 to 25 years, and 350 aged 26 to 60 years. The individuals over 18 years old were blood donors; those under 18 years were children who attended hospital for surgical purposes (tonsillectomy, circumcision, orthopaedic surgery). Individuals in group 2 were investigated for serological markers of HBV, HCV and HDV infection to define the temporal patterns of seroconversion in various age groups. HBV and HCV serological markers were determined in all sera, and HCV markers in a subgroup of 506 randomly selected individuals aged 6 months to 60 years. Group 3 included 477 pairs of infants 3 months old and their respective mothers, who attended health care centres in Tunis district for primary vaccination with diphtheria–tetanus–pertussis–polymyxin vaccine. This group was investigated to evaluate perinatal transmission of HBV.

Serological assays
HBsAg and antibodies to HCV (HCVAb) were tested for by the 3 centres involved in the study in Tunisia, using either commercial enzyme-linked immunosorbent assay (ELISA) kits from Sanofi Diagnostics Pasteur and Organon Teknika for HBsAg (Monalisa™ AgHrs 2nd generation, Hepatostika™ HBsAg uniform II) and from Sanofi Diagnostics Pasteur and Ortho Diagnostic Sys-
From Organon Teknika (Hepanostika = antiHBc and ed for in the Pasteur Institute in Tunis. Antibodies to Pasteur). Sera reacting with 2 antigens were HBs, MonolisaTM AcHBc, MonolisaTM HBeAg/Ab) and HDV antigen (HDVAg) using commercial ELISA kits from Sanofi Diagnostics Pasteur (MonolisaTM anticorps HBs, MonolisaTM AcHBc, MonolisaTM HBEAg/Ab) and from Organon Teknika (Hepanostika™ antiHBc and Hepanostika™ antiHBs). Sera positive for at least one HBV serological marker were investigated for immunoglobulin G antibodies to HDV (HDVAb) and for HDV antigen (HIVAg) using commercial ELISA kits from Sanofi Diagnostics Pasteur (Deltassay™ Ag and Deltassay™ Ac-Ab-Ak).

Statistical analysis

Categorical variables were compared using Fisher's exact \( \chi^2 \) test.

Results

Prevalence of HBV carriers and HCV

HBsAg was detected in 6.5% (51) of the people in group 2 and in 6.3% (2010) of the young military recruits (group 1), with a large geographical variation, ranging from 3% to 13% in the 23 governorates of Tunisia. Overall, the highest prevalences were found in southern regions of the country, with a progressive increase in seroprevalence from north to south: 5.5% in northern Tunisia, 7.5% in the central region, and 9.6% in the south (P=0.001). Among 1174 military recruits who were HBsAg positive at the beginning of their military service, 1068 (91%) had become HBsAg negative at a second serological examination 6 months later. The prevalence of HCV infection was 0.4% (3/785 tested) in group 2 (all ages included) and 0.2% (19/ 9782 tested) in group 1 (men aged 20-25 years). The prevalence of HCV infection, in group 1 subjects, was higher (0.31%, 7/2230) in the north-west of the country and 0.15%, 12/7894 in the south (P=0.001). Among 1174 military recruits who were HBsAg positive at the beginning of their military service, 1068 (91%) had become HBsAg negative at a second serological examination 6 months later. The prevalence of HCV infection was 0.4% (3/785 tested) in group 2 (all ages included) and 0.2% (19/ 9782 tested) in group 1 (men aged 20-25 years). The prevalence of HCV infection, in group 1 subjects, was higher (0.31%, 7/2230) in the north-west of the country than elsewhere (0.15%, 12/7894), although the difference was not statistically significant (P=0.08).

Sero-prevalence of HBV, HCV, and HDV according to age

In group 2 subjects, the overall prevalence of HBV infection was 37.5%: 295/785 individuals expressed at least one serological marker for HBV. The HBV infection rate increased with age: there was a steep increase in infancy and adolescence and a mild increase between 20 and 45 years, followed by an almost steady state around 50% in those aged over 45 years (Figure). The rate of HBV infection was slightly higher in men (35.9%, 158/442) than in women (35.7% 137/383), but the difference was not statistically significant (P=0.24). The prevalences of HBsAg were 6.7% and 6.2% in men and women respectively. Among the blood donors, HBsAg was detected in 43.5% of sera, associated with HBsAb in 33-35% of cases. HBcAb was the sole marker in 4-5% of cases. HDVAg was detected in 1-5% of specimens (10 of 650 sera from group 2), which were also HBsAg positive. HDVAg was more frequently detected, in 105 of 650 sera (16.1%).

Vertical and perinatal transmission of HBV

Only 2 infants of the 477 examined (group 3) were HBsAg positive at 3 months of age: one was born to a mother who was HBsAg and HBeAg positive, and the other child was born to an HBV seronegative mother. Thus, the prevalence of vertical and perinatal transmission in the first 3 months of life was 0.4%. Among the 477 mothers studied, 18 (3.8%) were HBsAg positive; only one was positive for both HBsAg and HBeAg (the woman who had transmitted the infection to her child). The remaining 17 women were HBsAg positive, with no evidence of HBV transmission to the child.

Discussion

Our study confirmed the high prevalence of HBV infection in Tunisia and the need for an effective preventive strategy. Overall, 37.5% of Tunisians were infected with HBV (i.e., expressed at least one viral or antibody marker) and this proportion reached 50% for people aged over 40 years. Vertical and perinatal transmission of HBV does not appear to play a major role in Tunisia; horizontal transmission, mainly in infancy and adolescence, appears to be the major mode of infection. Compared to sub-saharan Africa, the active period for HBV transmission in Tunisia seems to be in slightly older age groups—children aged over 5 years, teenagers, and young adults. Most infected persons convert before 20 years of age and, considering the sexual behaviour of this age group in the country, this suggests that sexual transmission does not play a dominant role. The routes of transmission to infants are presently unknown. Possible routes include intrafamilial or school close contacts, or parenteral transmission via practices like scarification, tattooing, and traditional circumcision. These latter practices, although decreasing throughout the country, still exist in regions of lower socioeconomic level, particularly in the south of the country, which could explain the higher prevalence of HBsAg positivity found in these regions. However, it is worth noting that the rate of HBsAg positivity may vary within a wide range in the same region (range 3% to 13.5%). This prevalence variability may reflect more intense viral transmission due to some particular characteristics of the HBV strains or to the genetic background of the local population.

It is generally assumed that chronic carriers of the virus are the principal reservoir of HBV. Our results
showed that 90% of young adults aged around 20 years (group 1) who were positive for HBsAg expressed this marker transiently and spontaneously seroconverted to HBsAg negativity 6 months later. These results suggest that asymptomatic infection, in young people transiently harbouring the virus, may make an important contribution to HBV transmission. This pool of young people, with current asymptomatic HBV infection, could contribute to the transmission of the virus either within their households or to their sexual contacts outside the dwelling.

Hepadnavirus infection is also quite common in Tunisia; 44% of HBsAg negative persons had been superinfected with HDV. HDV infection occurs later than HBV: we could not detect any HDV seropositivity before the age of 13 years, despite the fact that about 20% of children in that age group had already been infected with HBV. Later, HDV infection progressed with age similarly to HBV infection, suggesting similar modes of transmission. The fact that HDV superinfection occurred mainly after 20 years of age, whereas HBV infection occurred before that age, is paradoxical if we consider that the biology of the 2 viruses is closely linked. This suggests that the transmission mechanisms in the 2 age groups were different: before the age of 20 years, the factors accounting for the high rate of HBV transmission do not involve HDV. After 20 years of age, sexual intercourse may be an important route for HDV transmission. Strikingly, HDV superinfection in HBsAg negative adults appears to be quite common since, after the age of 30 years, more than 50% of such people are superinfected.

Compared to HBV, the seroprevalence of HCV in the Tunisian population is low—0.4%, similar to that reported in most European countries (0.2-0.8%; KAMEL et al., 1989; CHEN et al., 1989; KUO et al., 1989; KUO et al., 1989; CONTRES et al., 1991) and below that reported in other regions of the world (0.8-1.4%; KAMITSUZAKA et al., 1989; SHIRISHIA et al., 1989; CHEN et al., 1990; KIEW et al., 1990; FROMMEI et al., 1993). For unknown reasons, very high prevalences, over 10%, have been reported in Egypt (KAMEL et al., 1992; DARWISH et al., 1993). The contrast between the high prevalence of HBV and HDV infections in Tunisia and the relatively low prevalence of HCV infection could indicate a different distribution of the viruses, lower infectivity of HCV, and different routes of transmission. In fact, the 2 infections do show slight differences in their geographical distribution: HBV is more frequent in the south and the mid-west of the country, whereas HCV infection appears to occur more frequently in the north-west. Moreover, while HBV-infected adults are mainly transmitted throughout childhood and adolescence, HCV infection seems to occur later, as suggested by other studies (CHEN et al., 1990; SCOTT et al., 1992; FROMMEI et al., 1993).

Due to the considerable socioeconomic and population structure, it is likely that the results reported in this study for Tunisia are also relevant to other North African countries. Introduction of mass HBV vaccination and, hopefully, in the future an HCV vaccine, will help to control the impact of the hepatitis virus B and C in this country.
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