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Heterogeneity of hepatitis B transmission in Tunisia: Risk factors for infection and chronic carriage before the introduction of a universal vaccine program☆

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ABSTRACT

A population-based sero-epidemiological study enrolled 9486 volunteers in two governorates, Béja in the north and Tataouine in the south of Tunisia, in order to assess the magnitude of HBV transmission heterogeneity between the north and the south and within the same governorate, as well as the risk factors associated with infection and chronic carriage. *Results:* The overall prevalence of anti-HBc, HBsAg and chronic carriage was 28.5, 5.3 and 2.9%, respectively. Significant differences were observed between the two governorates according to anti-HBc (32.1% in Béja and 27.8% in Tataouine; $p = 0.005$) and HBsAg prevalence (4.2% in Béja and 5.6% in Tataouine; $p = 0.001$). Significant differences were noticed between districts revealing important heterogeneity in HBV transmission within the same governorate (HBsAg ranged from 12 to <2% within the same governorate). At the individual level, the presence of a family member infected with HBV, scarification practices, needle practices in the Primary Care Center and gender (male) significantly increased the risk of anti-Hbc, HBsAg positivity and chronic carriage of infection while existence of sanitation in the house was found to be protective. The basic reproductive number and the force of infection confirmed the heterogeneity of transmission. Horizontal transmission within the family explains hyperendemic clusters in Tunisia.

1. Introduction

In Tunisia, Hepatitis B represents a major public health problem because of its high morbidity and mortality rates. Indeed, hepatitis B along with tuberculosis and leishmaniasis account for 75% of compulsory notifiable diseases [1]. According to previous studies in Tunisia, prevalence of HBsAg and HBV infection range from 6.3 to 7.8% and 37.5 to 48.5%, respectively [2–4]. These prevalences confirm the intermediate HBV endemicity in this country. Males have been shown to have higher HBV infection rates (current and/or past) than females [2–4]. Not surprisingly, a young population (under 20) has been shown to have a higher HBsAg prevalence than an adult population [2–4]. Previous evidence suggested that endemicity might be higher in southern Tunisia with a chronic carriage prevalence exceeding 15% in some villages [2–4]. This hypothesis has never been tested on a population-based representative sample. Factors discriminating populations at higher risk have not been investigated. In addition, the chronic carriage of HbsAg has not been evaluated over a period longer than 6 months. The incidence of infection among susceptibles has also not been evaluated in Tunisia.

This study is the first performed on a representative community-based sample that included the northern and the southern parts of Tunisia. We hypothesized that, in addition to the north-south-gradient, there would also be a strong variation in transmission within each part of Tunisia. Indeed, risk factors might be related to behavioural and demographic characteristics of the family, whatever its geographic location. Furthermore, the study was undertaken just before the implementation of the universal HBV vaccination in Tunisia, so that the study will assess the situation before the start of this control strategy and provide important information for policy makers on its value. The information gained might help to further fine tune the control program by permitting the control strategy to be modified according to local needs.

This study aimed to compare seroprevalence of hepatitis B markers in two regions, one in the north and one in the south of the country, and to assess risk factors associated with infection and chronic carriage. The method used was a community-based survey

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utilizing house to house visits to a representative sample of eligible families. In addition, this study assessed the incidence of the infection using a representative cohort of susceptibles as well as the force of infection (FOI) according to the level of endemic city.

2. Methods

2.1. Design

A sero-epidemiological population-based cross-sectional study ($n = 9486$) was carried out during 1996, before the introduction of the universal vaccine program, in two governorates: Béja in the north and Tataouine in the south of Tunisia. The subgroup of HBsAg positives during the first measurement ($n = 502$) was resampled 3 years later to properly assess the chronic carrier status of this marker. Furthermore, a representative subsample (Dhiba and Rogba) of seronegative individuals for all markers ($n = 291$) was also reassessed 3 years later to evaluate the mean incidence of HBV infection in the study area.

2.2. Study population

The study population included two governorates: Béja in the north and Tataouine in the south. In Béja, three representative villages, one urban (Medjez El Bab Ouest), one sub urban (Khniguet Eddhene) and one rural (Bir Elleuch), were included. In the governorate of Tataouine, all villages covering rural, sub-urban, urban and also villages of Berber origin were included. A random sample representative of each village was selected using a simple two-stage cluster sampling: the first stage is the village; the second stage is the family. All subjects of selected families were asked if they were willing to be enrolled in the study. Table 1 shows the number of individuals sampled per village and the parameters tested in their blood.

2.3. Data collection

Data collection was performed by door-to-door visits to all houses within the study area. After oral consent was given, a pre-tested structured questionnaire was administered by trained interviewers to collect three types of information: (i) description of the dwelling (e.g. type of wall, type of roof); (ii) socio-economic description of the family (e.g. number of rooms used by the family, type of water supply, use of electricity, health care accessibility); (iii) information about each family member (e.g. date of birth, gender, family status, education level, behaviours that constitute potential risk factors for HBV infection: traditional circumcision, tattoo-age, scarification.). Subjects who consented to be enrolled in the study provided a blood sample for serological testing.

Sera were tested for hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc). All sera were tested for HBsAg and anti-HBc. In order to assess the prevalence of HBV chronic carriage, all HBsAg positive individuals were resampled in 1999, 3 years after the date of the first sample. Sera were tested for HBsAg using commercially available kits for enzyme linked immunosorbant assay-III (hepanostika HBsAg and hepanostika HBc antibody—Biomerieux).

Individuals were categorized into two different HBV infection groups: HBV-positive and HBV-negative groups. The HBV-positive group was comprised of subjects who tested positive for any HBV marker, and the HBV-negative group tested negative for all markers. The HBV-positive group was divided into tree subgroups: anti-HBc-positives, HBsAg positive and chronic carriers (HBsAg positives for whom this antigen remained positive during the second sampling). The study area was divided into three areas according to their endemicity level: hyperendemic with more than 8% of the population being HBsAg positive; meso-endemic with 2–7% of the population being HBsAg positive and hypo-endemic area with less than 2% of the population being HBsAg positive.

2.4. Data analysis

Demographic, socio-economic information and HBV markers test results were merged in the same database using Oracle release 6 software. All the entered data was cleaned by comparing electronic information against source documents. SPSS version 13.0 was used to perform the statistical analysis of data.

Prevalence of HBV infection was estimated via sample proportions, and exact binomial computation was used in estimating 95% confidence intervals [CIs]. All prevalences were standardized by age to allow comparisons between districts. Mean values (±SD) for age were compared between the HBV groups using the ANOVA test. The Chi-square test was used to evaluate gender distribution differences. After adjustment for age, an analysis of the relationship between HBV groups, demographic characteristics, and identified risk factors was conducted. A multivariate logistic regression model was also developed. All variables were initially included in the model. Possible interactions between age, gender and other variables were also explored. Only statistically significant demographic and exposure characteristics were retained in the final multivariate logistic model. Significance values below the 0.05 level were considered significant.

The force of infection (FOI), defined as the instantaneous per capita rate at which susceptible individuals acquire infection [5], was estimated by fitting a polynomial function to observed data using the loglikelihood method by Matlab 7.7 software [6]. The basic reproductive number R_0 was estimated as proposed by Anderson and May by the reverse of the proportion of susceptible ($1/x^*$) [7].

3. Results

3.1. Description of participants

In total 9486 subjects were enrolled in the study of which 2223 were from Beja, and 7235 from Tataouine. The mean age of HBV tested subjects was 26.3 ± 20.7 years (min 0.02 max 95.8), while 57.6% were female, 32.4% were illiterate, and only 12.5% had sanitation in their houses. 80 of the 246 HBsAg positive patients during the first measurement were not evaluated 3 years later (32.5%).

3.2. HBV markers prevalence

The mean age of anti-HBc, HBsAg subjects and chronic carriers was 36.2 ± 22.6 years, 26.9 ± 19.1 years, and 23.9 ± 16.4 years, respectively. The male to female ratio was 0.79 for anti-HBc subjects, 1.06 for HBsAg subjects and 1.09 for chronic carriers. The overall prevalence of anti-HBc, HBsAg and chronic carriage was 28.5% $CI_{95\%}$ [27.6–29.4%], 5.3% $CI_{95\%}$ [4.8–5.8%] and 2.9% $CI_{95\%}$ [2.6–3.2%], respectively. A Significantly higher prevalence is noted in males compared to females: 29.6% $CI_{95\%}$ [27.6–29.4%] vs. 27.7% $CI_{95\%}$ [26.5–28.9%] ($p = 0.047$) for anti-HBc; 6.4% $CI_{95\%}$ [5.6–7.2%] vs. 4.5% $CI_{95\%}$ [3.9–5.1%] ($p < 10^{-3}$) for HBsAg and 3.6% $CI_{95\%}$ [3.4–3.7%] vs. 2.4% $CI_{95\%}$ [2.0–2.8%] ($p = 0.001$) for chronic carriers. Prevalence of anti-HBc and HBsAg increases significantly with age globally for both males and females ($p < 10^{-3}$).

The distribution of HBV markers per governorates and districts is illustrated in Table 1. After standardisation per age significant differences were observed between the two governorates according to anti-HBc prevalence (32.1% $CI_{95\%}$ [28.9–32.7%] in Béja and 27.8% $CI_{95\%}$ [26.8–28.8%] in Tataouine; $p = 0.005$) and HBsAg prevalence (4.2% $CI_{95\%}$ [3.2–4.8%] in Béja in the north and 5.6% $CI_{95\%}$ [5.2–6.2%]

Table 1
Crude and standardized HBV infection prevalences in the study area.

Governorate Village (*N*)	Crude prevalences			Standardized prevalences		
	Anti-HBc+ *N* (%)	HBsAg + *N* (%)	Carriers *N* (%)	Anti-HBc+ (%)	HBsAg + (%)	Carriers (%)
Béja (North) (2223)	688 (30.8)	89 (4.0)	55 (2.5)	32.1%	4.2%	2.6%
Mjez Elbab Ouest (622)	191 (30.5)	12 (1.9)	6 (0.9)	31.0%	2.1%	1.2%
Bir el euch (732)	144 (19.6	12 (1.6)	8 (1.1)	20.5%	1.8%	1.1%
Khniguet eddhene (869)	353 (40.5)	65 (7.5)	41 (4.8)	41.7%	7.7%	4.9%
Tataouine (South) (7235)	2017 (27.8)	413 (5.7)	213 (3.0)	27.8%	5.6%	2.8%
Ajej (412)	28 (6.8)	0 (0.0)	0 (0.0)	6.6%	0.0%	0.0%
Bir tlethine (939)	279 (29.7)	36 (3.8)	13 (1.4)	28.8%	3.8%	1.3%
Chnenni (377)	59 (15.6)	1 (0.3)	0 (0.0)	16.6%	0.3%	0.0%
Rogba (545)	102 (18.7)	6 (1.1)	3 (0.6)	18.7%	1.2%	0.5%
Ras el oued (440)	242 (54.6)	70 (15.9)	33 (8.0)	55.9%	16.1%	7.7%
El ouaha (407)	56 (13.8)	5 (1.2)	0 (0.0)	14.9%	1.2%	0.0%
Dhiba(1180)	766 (64.3)	240 (20.3)	142 (12.9)	64.1%	19.6%	12.0%
Rmada est (597)	160 (26.8)	26 (4.4)	12 (2.1)	27.5%	4.3%	2.0%
Gheriani (284)	65 (22.8)	14 (4.9)	3 (1.1)	23.5%	5.2%	1.1%
El morra (74)	0 (0.0)	0 (0.0)	0 (0.0)	0.0%	0.0%	0.0%
Gsar mrabtine (200)	26 (13.0)	3 (1.5)	1 (0.5)	13.0%	1.2%	0.5%
Tlelet (824)	46 (5.6)	3 (0.4)	1 (0.1)	5.9%	0.4%	0.1%
Greguer (296)	58 (19.5)	5 (1.7)	1.4 (1.4)	19.6%	1.6%	1.3%
Oued el gamh (661)	130 (19.6)	4 (0.6)	1 (0.2)	19.7%	0.7%	0.1%
Total	2697 (28.5)	502 (5.3)	268 (2.9)			

in Tataouine in the south; $p = 0.001$). No significant differences were noted according to chronic carriage prevalence between the two governorates (2.6% $CI_{95\%}$ [1.9–3.1%] in Béja vs. 2.8% $CI_{95\%}$ [2.6–3.4%] in Tataouine). When the analysis was refined at the subgovernorate level, significant differences were noted between districts according to these three markers (all p values $<10^{-3}$). Ras el oued and Dhiba (in the south) showed a higher prevalence for all HBV markers than the other districts. If HBV chronic carriage prevalence (7.7 and 12.0%, respectively) is considered, these two districts are classified as areas of high endemicity. Khniguet eddhene (in the north) and Rmada est (in the south) show an HBV chronic carriage prevalence of 4.9 and 2.0%, respectively, and can then be classified as areas of intermediate endemicity. All other districts have HBV chronic carriage prevalence less than 2% and are thus classified as areas of low endemicity. Interestingly, the relative proportion of carriers among HBsAg positive subjects differ significantly ($p < 10^{-3}$) between districts, and ranges from 30 to 90% (Fig. 1). Not surprisingly, the age-distribution of HBsAg, anti-HBc, and chronic carriage prevalence increased as endemicity decreased. The median age of all HBV infection markers was lower in hyperendemic areas as compared to intermediate and hypo-endemic ones. The median age for anti-HBc positive subjects was 24.3 years, 30.8 years, and 40.0 years ($p < 10^{-3}$); for HBsAg positive subjects, was 16.9 years, 23.0 years, and 29.9 years ($p < 10^{-3}$); and for chronic carriers, was 14.7 years, 24.7 years and 29.8 years ($p < 10^{-3}$) for hyperendemic regions, intermediate endemic regions, and low endemic regions ($p < 10^{-3}$), respectively. Similarly, the age at which half the population have been infected decreased significantly from low (60 years) to intermediate (40 years) and high endemic regions (10 years) (Fig. 2a). The age distribution of anti-HBc and chronic carriage showed different patterns according to endemicity (Fig. 2b). In a hyperendemic area, chronic carriage increased quickly and saturated after the age of 20 years. On the other hand, in meso- and hypo-endemic areas, a steady increase of chronic carriage with age was noted.

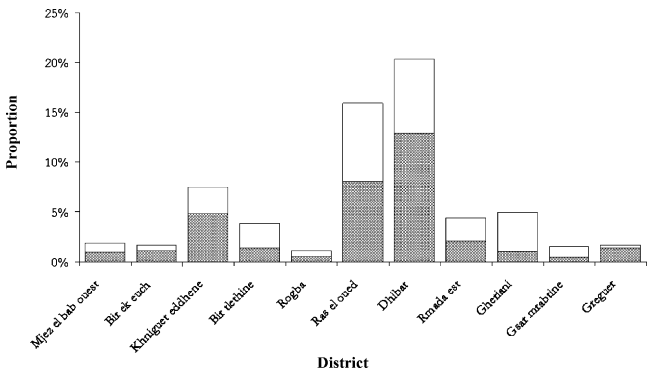

Fig. 1. Relative proportion of carriers (dashed area) among AgHBs positive subjects (total bars).

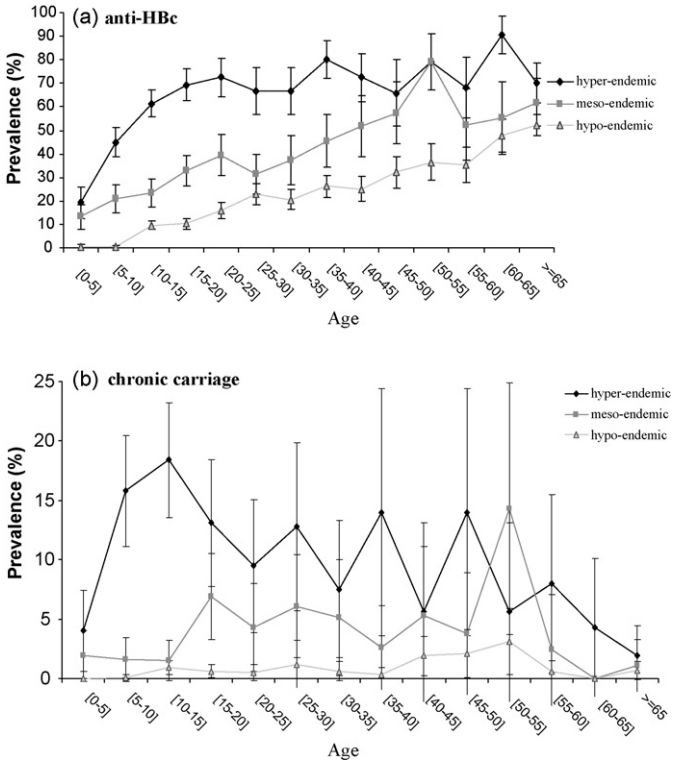

Fig. 2. Age prevalence of anti-HBc (a) and chronic carriage (b) according to endemicity level in the study area.

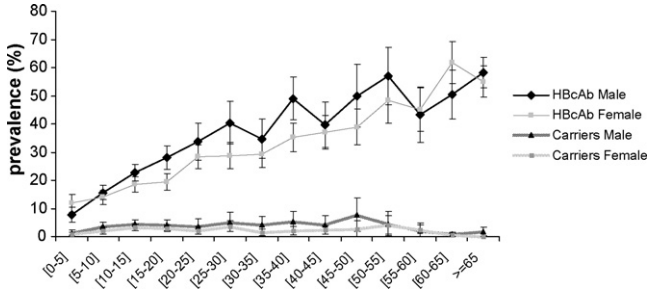

Fig. 3. Age prevalence of anti-HBc and chronic carriage by gender in the study area.

HBsAg and HBV infection showed a higher prevalence in males before 55 years (Fig. 3).

3.3. HBV infection incidence

In total, a cohort of 291 susceptibles was included to evaluate the HBV incidence: 75 in Dhiba (hyperendemic region) and 216 in Rogba (hypo-endemic region). At baseline in 1996, they were seronegative for all markers and they were retested for HBV infection markers 3 years later. They did not receive any HBV vaccine between the 2 tests. Out of the total sample of the cohort, 15 in Dhiba and 6 in Rogba seroconverted corresponding to a cumulative incidence during 3 years of 20.0% $CI_{95\%}$ [10.95–29.05%] and 2.8% $CI_{95\%}$ [0.60–5.00%] in Dhiba and Rogba, respectively, leading to a mean annual incidence of infection of 6.67% $CI_{95\%}$ [3.65–9.70%] and 0.93% $CI_{95\%}$ [0.20–1.67%] in these two villages ($p < 10^{-3}$).

3.4. Risk factors analysis

The first part of the analysis is related to the study of environmental, demographic and behavioural risk factors at the individual level. Bivariate analysis revealed that education level, past history of scarification, needle practices in the Primary Care Centre (PCC), gender, existence of sanitation in the house, and family scarification practices were significantly associated with HBV infection and chronic carriage (Table 2).

By multivariate analysis, family scarification practices, needle practices in the PCC and gender were significantly associated with anti-Hbc positivity (AOR equal to 2.15 $CI_{95\%}$ [1.85–2.49], 1.64 $CI_{95\%}$ [1.36–1.97] and 1.26 $CI_{95\%}$ [1.12–1.42], respectively). The same risk factors were found for HBsAg positivity (AOR equal to 2.36 $CI_{95\%}$ [1.60–3.00], 1.85 $CI_{95\%}$ [1.24–2.77] and 1.53 $CI_{95\%}$ [1.23–1.90], respectively) and chronic carriage (AOR equal to 2.85 $CI_{95\%}$ [2.10–3.86], 2.37 $CI_{95\%}$ [1.33–4.19] and 1.37 $CI_{95\%}$ [1.02–1.83], respectively). Lack of sewage in the house was found to be protective against anti-HBc (AOR equal to 0.49 $CI_{95\%}$ [0.37–0.65]), and HBsAg positivity (AOR equal to 0.08 $CI_{95\%}$ [0.02–0.31]). No significant association between HBV subgroups and household size was noted (Table 3).

Table 2
Risks associated with HBV infection and chronic carriage by bivariate analysis.

Factors	OR	95% CI	*p* value
Anti-Hbc (positive/negative)			
Illiterate (yes/no)	2.34	[2.13–2.51]	$<10^{-3}$
Past history of scarification (yes/no)	1.74	[1.43–2.12]	$<10^{-3}$
Needles in the PCC (yes/no)	1.68	[1.43–1.97]	$<10^{-3}$
Sanitation (yes/no)	0.66	[0.57–0.77]	$<10^{-3}$
Gender (male/female)	1.10	[1.02–1.20]	0.04
Scarification practice in the family	2.00	[2.13–2.51]	$<10^{-3}$
HBV chronic carriage (yes/no)			
Scarification (yes/no)		[1.20–2.91]	$<10^{-3}$
Needles in the PCC (yes/no)		[1.44–4.29]	$<10^{-3}$
Sanitation		[0.16–0.54]	$<10^{-3}$
Gender (male/female)		[1.19–1.93]	0.04
Scarification practice in the family		[2.02–3.63]	$<10^{-3}$

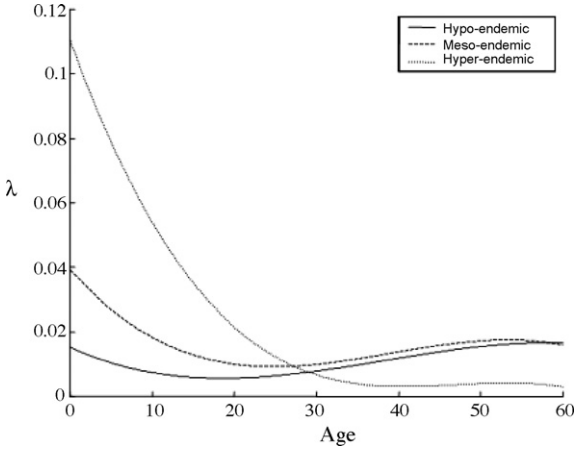

Fig. 4. The force of HBV Infection by age and endemicity level.

The second part of the analysis attempted to assess the importance of transmission within the family as a risk factor to acquire infection for the individual. We concentrated on the study of non-sexual close contact risks. Therefore, we evaluated the risk of HBV infection of the individual due to: (i) HBV chronic carrier mother, (ii) HBV chronic carrier brother/sisters(s), and (iii) and HBV chronic carrier father.

Individuals having a carrier mother are about three times more likely to be anti-HBc positive (AOR = 2.97 $CI_{95\%}$ [1.86–4.75]), 10 times more likely to be HBsAg positive (AOR = 10.64 $CI_{95\%}$ [6.23–17.82]) and six times more likely to be chronic carriers (AOR = 5.65 $CI_{95\%}$ [3.09–10.33]). Those having HBV chronic carrier brother(s) or sister(s) are at high risk of HBV infection (AOR equal to 11.60 [8.35–16.12] for anti-HBc and 13.61 $CI_{95\%}$ [8.78–21.07] for HBsAg) and chronic carriage (AOR = 24.73 $CI_{95\%}$ [13.56–45.12]). Risks associated with a chronic carrier father are equal to 2.59 $CI_{95\%}$ [1.71–3.93] for anti-HBc positivity, 6.00 $CI_{95\%}$ [3.56–10.13] for HBsAg positivity and 2.67 $CI_{95\%}$ [1.43–5.00] for being a chronic carrier (Table 4). A family having a HBV chronic mother is at high risk of having multiple (more than 2) HBV carriers (AOR = 35.79 $CI_{95\%}$ [17.56–72.94]; $p < 10^{-3}$). The risk of multiple HBV carriers associated with HBV chronic father is 19.40 $CI_{95\%}$ [7.65–49.28] ($p < 10^{-3}$). Scarification practices in the family multiplies the risk of multiple HBV carriers by 4.20 $CI_{95\%}$ [2.25–7.84] ($p < 10^{-3}$).

3.5. Transmission dynamics

The mean age at infection was 30.4 in hyperendemic versus 34.5 in meso-endemic and 41.5 in hypo-endemic areas. Likewise, the estimation of the proportions of those susceptible was correlated with different endemicity levels for HBV transmission. The basic reproductive number was 1.26, 1.55 and 2.64 in hypo-, meso- and hyper-endemic areas respectively (Table 5).

The force of infection (FOI) was significantly higher in the hyperendemic areas compared to meso- and hypo-endemic ones, particularly during childhood and early infancy. By the age of ~30 years, the transmission seems to be similar among the three groups and slightly increases among meso- and hypo-endemic areas for adults. In hyperendemic area, the FOI peaked in infancy and early childhood, declined rapidly with age, dropped to a low level and remained constant after at the age of 30 years (Fig. 4).

Table 3
Multivariate analysis by logistic regression of HBV markers and chronic carriage risk factors.

Factors	AOR[a]	95% CI	*p* value
Anti-Hbc (positive/negative), *N* = 5845			
Needles in the PCC (yes/no)	1.64	[1.36–1.97]	$<10^{-3}$
Sewage (yes/no)	0.49	[0.37–0.65]	$<10^{-3}$
Scarification practice in the family	2.15	[1.85–2.49]	$<10^{-3}$
Gender (male/female)	1.26	[1.12–1.42]	$<10^{-3}$
HBsAg (positive/negative), *N* = 5830			
Needles in the PCC (yes/no)	1.85	[1.24–2.77]	0.003
Sewage (yes/no)	0.08	[0.02–0.31]	$<10^{-3}$
Scarification practices in the family	2.36	[1.60–3.00]	$<10^{-3}$
Gender (male/female)	1.53	[1.23–1.90]	$<10^{-3}$
HBV chronic carriage (yes/no), *N* = 5851			
Needles in the PCC (yes/no)	2.37	[1.33–4.19]	0.003
Scarification practice in the family	2.85	[2.10–3.86]	$<10^{-3}$
Gender (male/female)	1.37	[1.02–1.83]	0.035

[a] Adjusted for age.

4. Discussion

The overall prevalence of anti-HBc, HBsAg and chronic carriage was 28.5, 5.3 and 2.9%, respectively. Significant differences were observed between the two governorates and between districts revealing important heterogeneity in HBV transmission within the same governorate. Analysis of risk factors demonstrate that the presence of a family member infected with HBV, scarification practices, needle practices in the Primary Care Center and gender (male) significantly increased the risk of anti-Hbc, HBsAg positivity and chronic carriage of infection while existence of sanitation in the house was found to be protective.

Despite the wealth of information provided by previous research conducted in Tunisia, these studies suffered from several methodological shortcomings [2–4]. They were limited either by the hospital-based character of samples, or by the fact that they were restricted to some risk groups or had a narrow age range, such as military recruits. Therefore, their findings cannot be generalized to the total population. Furthermore, the chronic carriage of the virus in previous studies was rarely assessed by two consecutive measurements at a time interval greater than 6 months. Moreover, few studies attempted to properly address with representative samples the comparison of patterns of infection and chronic carriage in northern and southern parts of the country. The risk factors for infection and chronic carriage are not fully understood. In many developing countries, the relative contribution of various routes of HBV infection have not been defined in population-based studies [8]. Moreover, in a low socio-economic setting, horizontal transmission of HBV has been reported and needs to be verified [9]. The current study presents the first data on seroprevalence, incidence, and associated risk factors of HBV infection and chronic carriage in a large population-based study. Our data were complete, plausible,

Table 4
Multivariate analysis by logistic regression of HBV markers and chronic carriage risk factors in siblings.

Factors	AOR[a]	95% CI	*p* value
Anti-Hbc (positive/negative), *N* = 2895			
HBV chronic carrier mother	2.97	[1.86–4.75]	$<10^{-3}$
HBV chronic carrier brother/sisters(s)	11.60	[8.35–16.12]	$<10^{-3}$
HBV chronic carrier father	2.59	[1.71–3.93]	$<10^{-3}$
Needles in the PCC (yes/no)	1.56	[1.16–2.09]	0.003
Sewage (yes/no)	0.54	[0.35–0.83]	0.005
Scarification practice in the family	2.06	[1.60–2.66]	$<10^{-3}$
Gender (male/female)	1.21	[1.00–1.46]	0.045
HBsAg (positive/negative), *N* = 2892			
HBV chronic carrier mother	10.64	[6.23–17.82]	$<10^{-3}$
HBV chronic carrier brother/sisters(s)	13.61	[8.78–21.07]	$<10^{-3}$
HBV chronic carrier father	6.00	[3.56–10.13]	$<10^{-3}$
Gender (male/female)	1.68	[1.10–2.57]	0.017
HBV chronic carriage (yes/no), *N* = 2906			
HBV chronic carrier mother	5.65	[3.09–10.33]	$<10^{-3}$
HBV chronic carrier brother/sisters(s)	24.73	[13.56–45.12]	$<10^{-3}$
HBV chronic carrier father	2.67	[1.43–5.00]	0.002

[a] Adjusted for age.

Table 5
Force of infection estimation per endemicity level.

Area	Mean age at infection	Force of infection					x^*	R_0
		Constant	Best fit (degree 3)					
			a_3	a_2	a_1	a_0		
Hypo-endemic	40.99	0.00946	−3.6E−07	4.1E−05	−0.00116	0.0152903	79.41	1.26
Meso-endemic	34.53	0.01825	−7.2E−07	8.4E−05	−0.00286	0.0392864	64.71	1.55
Hyper-endemic	30.40	0.04203	−1.1E−06	0.00015	−0.00708	0.1103644	37.8	2.64

and in accordance with previously available information, supporting the overall validity of our study population.

The difference between the population included in the census and the blood sampled population is explained by absence or refusal of blood sampling on the day of visit. The difference between the blood sampled population and HBV tested population may be caused by the deterioration of the serum or lack of testing kits.

Moreover, according to the cultural habits in the study area, females are usually housekeepers or work around their homes and consequently more likely to be present in house to house surveys. Therefore, they seem to be over-represented in the sample after blood sampling. This is mainly due to the absence of males during blood sampling time, which corresponds to work time. These differences might potentially represent a selection bias and alter some characteristics of the initial population. To control this bias, all prevalences were standardized by age which permitted valid comparisons of HBV infection markers between districts. Similarly, the rate of HBsAg positive patients lost-to follow-up 3 years later (32.5%) is within the expected range for a prospective cohort study (~10% per year). It can be due to absence during the follow-up, death, immigration or refusal to be enrolled. This limitation might introduce a selection bias that could impact importance and geographic distribution of chronic carriage. However, estimated chronic carriage was coherent with prevalence of infection markers at baseline and the proportion of lost of follow-up did not differ significantly between the different villages. Therefore, we can rule out any significant effect on the validity of our estimations because of this limitation.

In the study sample, the gender and age representativeness of the HBV tested population was checked and seems to reproduce the age and gender distributions of the general population. Therefore, the study sample can be considered as representative of the target population with regard to the main study variables.

The 2.9% HBV chronic carriage prevalence overall found in this study corroborates previous estimations and confirms the intermediate endemicity of HBV infection in Tunisia. Significant difference in endemicity between districts and within the same district demonstrates the importance of the geographic heterogeneity of HBV transmission in Tunisia and corroborates findings described elsewhere [10–13].

The results regarding gender age-distribution of HBsAg and anti-HBc corroborate previous findings and confirm that males have higher HBV infection than females. The gender difference might reflect the increased frequency of high-risk behaviour, among men compared to women [14–16].

In the present study, risk factors of HBV infection and chronic carriage were gender, scarification practices, and needles in the Primary Care Center. Intramuscular (IM) injections [17] seem to play an important role in horizontal transmission of HBV via inadequately sterilized syringes used for iatrogenic IM injections in a community in which HBV was prevalent and IM injections were common [17,18]. Possible routes include intrafamilial or school close contacts, or parenteral transmission via practices like scarification, tattooing, and traditional circumcision was previously reported. These latter practices, although decreasing throughout the country, still exist in regions of lower socio-economic 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. This prevalence variability may reflect more intense viral transmission due either to some particular characteristics of the HBV strains or to the genetic background of the local population [4].

Environmental factors, like the existence of sanitation in the house, seem to be protective against anti-HBc and HBsAg positivity and reflect a higher socio-economic standard. Some studies have reported that HBV infection is more prevalent in rural areas and the increasing risk is related to environmental factors [11–13,19].

Intrafamilial horizontal transmission of HBV by coexistence of chronic HBV carriers with respect to the mother, father, brother or sister seems to be the most important route of transmission of HBV in Tunisia and explains hyperendemic microfoci of HBV transmission where a high clustering of infected cases and carriers is found in the same families. Child-to-child transmission was found to be more important than mother-to-child and father-to-child transmission. Many factors were reported to be associated with intrafamilial transmission of HBV infection [20–25]: sharing of various personnel and household articles such as a toothbrush, towel, handkerchief, clothing, razor, comb, or clothing [26]; ear-piercing and scarification [27]. Other studies have demonstrated that premastication of food to the children, a traditional habit frequent in rural Tunisia, is possibly an important factor in the family transmission of HBV [28]. Some other findings show that the risk of horizontal child-to-child HBV transmission is especially important during elementary school years [13,24,29]. The investigation of the mechanism leading to intrafamilial transmission is beyond the scope of our study.

The FOI reflects the degree of contact with potential for transmission between susceptibles and infected [30] and the type and frequency of exposure, as well as infection persistence [31]. The FOI was significantly higher in the hyperendemic areas compared to meso- and hypo-endemic ones particularly during childhood and early infancy [30–32]. These trends in FOI account for different transmissions routes in the different settings: familial versus sexual ones.

The sampling in the study area took place just before the introduction of a universal infant vaccination program against HBV which was included in Tunisian's national infant immunization calendar in 1996.

This study offers the opportunity to properly assess the impact of an HBV vaccination program by providing a valid evaluation of the epidemiologic situation just before the intervention. Further seroprevalence studies are in preparation now to monitor the efficacy of this program among the same communities.

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