Seroprevalence of hepatitis B infection in the Turkish population in Northern Cyprus

Zafer Kurugöl[1], Güldane Koturoğlu[1], Sadık Akşit[1], Tijen Özacar[2]

Northern Cyprus Study Team[a]

Departments of [1]Pediatrics and [2]Microbiology, Ege University Faculty of Medicine, İzmir, Turkey

[a]Sıdıka Kayımbaşoğlu, Sonay Özbalıkçı, Gülten Erçal, Emine Güllüelli, Figen Bakkaloğlu, Ertan Doğan, Hasan Garabli, Hüseyin Erçal and Cemal Mert

SUMMARY: Kurugöl Z, Koturoğlu G, Akşit S, Özacar T, Northern Cyprus Study Team. Seroprevalence of hepatitis B infection in the Turkish population in Northern Cyprus. Turk J Pediatr 2009; 51: 120-126.

This study was conducted to determine the seroprevalence of hepatitis B virus (HBV) infection in the Turkish population in Northern Cyprus. The secondary aim of this study was to assess the impact of the universal infant hepatitis B vaccination program, which started in 1998. A total of 600 persons 1 to 30 years old were selected for the study with cluster sampling. The information on sociodemographic characteristics was gathered for each participant and in 585 of them, hepatitis B surface antigen (HBsAg), anti-hepatitis B surface antigen antibody (anti-HBs) and anticore antibody (anti-HBc) were tested. The overall prevalence of anti-HBc and HBsAg carriage was 13.2% and 0.85%, respectively. Old age and low parental educational level were the major independent risk factors for HBV transmission. Seroprevalence of both anti-HBc and anti-HBs antibodies was similar in children 1-7 years of age. After 8 years of age, anti-HBc seroprevalence increased significantly with age, while anti-HBs prevalence decreased ($p<0.001$). Anti-HBc prevalence increased from 7.0% in children aged 1-7 years to 17.9% in persons aged 16-20 years. None of the children under 12 years of age were HBsAg-positive, while 1.9% of persons aged 16-20 years were HBsAg carriers. Anti-HBs seroprevalence exceeding 90% was found in the cohorts targeted by the routine hepatitis B vaccination program, whereas 36.4% of young adults aged 21-30 years were anti-HBs-positive. The study shows that universal infant hepatitis B immunization has a substantial impact on the immunity in children. However, prevalence of HBV infection is still high in adolescent and young adults in Northern Cyprus. Therefore, catch-up immunization for these groups will help to reduce hepatitis B transmission.

Key words: hepatitis B immunization, epidemiology, vaccine.

Hepatitis B virus (HBV) infection continues to be an important public health problem warranting high priority efforts for prevention and control. According to the most recent World Health Organization (WHO) estimate, approximately 30% of the world's population, i.e. about 2 billion people, have serological evidence of past or present HBV infection, and 360 million are chronically infected and at risk for HBV-related liver disease.[1] Approximately one-third of all cases of cirrhosis and half of all cases of hepatocellular carcinoma can be attributed to chronic HBV infection. HBV is estimated to be responsible for 600,000 deaths each year[1,2].

The frequency and patterns of HBV transmission vary markedly in different parts of the world. Approximately 45% of the world's population live in areas where the prevalence of chronic HBV infection is high (i.e., >8% of the population is HBsAg-positive); 43% live in areas of intermediate endemicity (i.e., 2% to 7% of the population is HBsAg-positive); and 12% live in areas of low endemicity (i.e., <2% of the population is HBsAg-positive)[3]. Turkey and Greece, which are two countries with a close relationship with Cyprus, have an intermediate level of HBV endemicity[4,5]. Cyprus is also considered to be an intermediate

endemicity area[6]. However, the epidemiology of HBV infection in the Turkish population in Northern Cyprus is not known because a community-based study on this disease has not been conducted in the population. We therefore conducted this study to determine the prevalence of HBV infection in the 1- to 30-year-old Turkish population in Northern Cyprus.

In 1992, WHO recommended the integration of hepatitis B vaccine into the national immunization programs of all countries by 1997[7]. As of 2004, more than 150 (78%) of 192 WHO member states had adopted universal childhood hepatitis B vaccination policies[8]. In the majority of the countries, vaccination coverage exceeded 80% within a few years of implementation[9] and the prevalence of HBV infection has been decreased dramatically[10-14]. For example, in Italy, the overall prevalence of chronic HBV infection declined by 27.5%[15]. Northern Cyprus introduced a universal infant hepatitis B vaccination program in 1998. The vaccination schedule adopted at the time was for babies to get the first dose at 2-3 months, the second dose at 3-4 months and the third dose at 6-9 months. The secondary aim of this study was to assess the impact of the mass vaccination program in reducing the prevalence of HBV infection.

Material and Methods

A prospective seroepidemiologic study of HBV infection was carried out in all provinces of Northern Cyprus in March 2006. The provinces were Lefkoşa, Gazimağusa, Girne, Güzelyurt and İskele. The study was approved by the Ethical Committee of Ege University Medical Faculty.

The study population included healthy subjects aged 1-30 years who were born in Cyprus. Subjects living in Northern Cyprus, but who had moved from Turkey were excluded from the study. The population of Turkish Cypriots was estimated as approximately 148,000 in 2003 by the United Nations and about 72,000 (48%) of this population were between 1 and 30 years of age.

The sampling method of 30 clusters recommended by the Expanded Programme on Immunization (EPI)/WHO for field studies[16,17] was used for selecting subjects of a pre-determined number in the rural and urban areas. The selection procedure was carried out by creating a cumulative list of community populations and selecting a systematic sample from a random start.

A total of 600 subjects 1 to 30 years old were selected for the study. In accordance with the population of provinces, 180 subjects from Lefkoşa, 150 subjects each from Gazimağusa and Girne and 60 subjects each from Güzelyurt and İskele were included in the study. A starting household was selected in each community by locating the ward's center, randomly selecting a house from a list of all houses falling along the line drawn from the ward center to the periphery in the chosen direction. The house was then examined to determine whether subjects of eligible age and sex lived there. Subsequently, the nearest household to the right was visited and the steps repeated until the desired number of persons was obtained. Only one individual from each family was selected in the study.

For each participant, a questionnaire was completed to provide information on socio-demographic characteristics and hepatitis B vaccination status. The information was obtained from the parents of the children and from the adults themselves. Immunization status of the study subjects was verified with their vaccination records, if available.

After written informed consent was obtained, blood samples were taken from each participant for seroprevalence analyses. Sera were stored at -20°C until tested at the Microbiology Laboratory of Ege University Faculty of Medicine. In order to differentiate the various possible stages of past or present HBV infection, a test combination of the measurement of anti-HBc, anti-HBs and HBsAg was used in this study. Hepatitis B surface antigen (HBsAg), antibodies to hepatitis B surface antigen (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc) were studied using a commercial ELISA method (RADIM S.p.a.; Rome, Italy).

Participants who were both anti-HBc- and anti-HBs-positive were classified as having been hepatitis B-exposed and possessing immunity (natural hepatitis B infection). Participants who were anti-HBc- and HBsAg-positive were classified as hepatitis B carriers. Persons who tested anti-HBc-negative but anti-HBs-positive were classified as most probably vaccinated.

Statistical analyses were performed using SPSS for Windows version 13.0. The association between the sociodemographic variables and prevalence of hepatitis B markers was

evaluated. Testing for statistical significance for univariate analysis was performed by the χ^2 test. A p value of <0.05 was considered as significant.

Multivariate analysis was performed using a logistic regression model containing the following independent variables: age, gender, region of residence, family income, education level and number of household members. The anti-HBc seroprevalence was fitted as the dependent variable in multiple regression to assess the influence of independent variables on anti-HBc seropositivity.

The number of persons in the household was categorized as "less than five persons" versus "five and more persons per household". Parental educational level was defined on the basis of the highest educational level achieved and was classified as group 1 (<8 years) or group 2 (≥9 years). The highest level achieved by one of the parents was used for children younger than 16 years.

Results

A total of 585 blood samples were analyzed (15 samples were excluded due to inadequate sample size or hemolysis). In 13.2% of the serum samples, there was evidence of a previous HBV infection, and 0.85% were HBV carriers (Table I). Anti-HBs was positive in 64.4% (n=377) of the serum samples. Of the 377 anti-HBs-positive participants, 322 (55.0%) were anti-HBc-negative, indicating that their antibody status was probably due to hepatitis B vaccination.

Table I. Hepatitis B Markers in the Study Population (n=585)

Marker	Number	Percentage	95% CI
HBsAg+	5	0.85	0.28-1.98
Anti-HBs+/anti-HBc+	55	9.4	8.7-11.4
Anti-HBc+	17	2.9	2.1-3.2
Anti-HBs+	322	55.0	50.1-59.9

CI: Confidence interval.

Seroprevalence of hepatitis B markers by age group is shown in Fig. 1. Seroprevalence of both anti-HBc and anti-HBs antibodies were similar in children 1-7 years of age. After 8 years of age, anti-HBc seroprevalence increased significantly with age, while anti-HBs prevalence decreased (p<0.001). Anti-HBc prevalence increased from 7.0% in children aged 1-7 years to 17.9% in persons aged 16-20 years (Fig. 1, Table II). None of the children under 12 years of age was HBsAg-positive, while 1.9% of persons in the 16-20 years of age group were HBsAg-positive. Anti-HBs seroprevalence was 90.7% in children aged 1-7 years, whereas 36.4% of young adults aged 21-30 years were anti-HBs-positive.

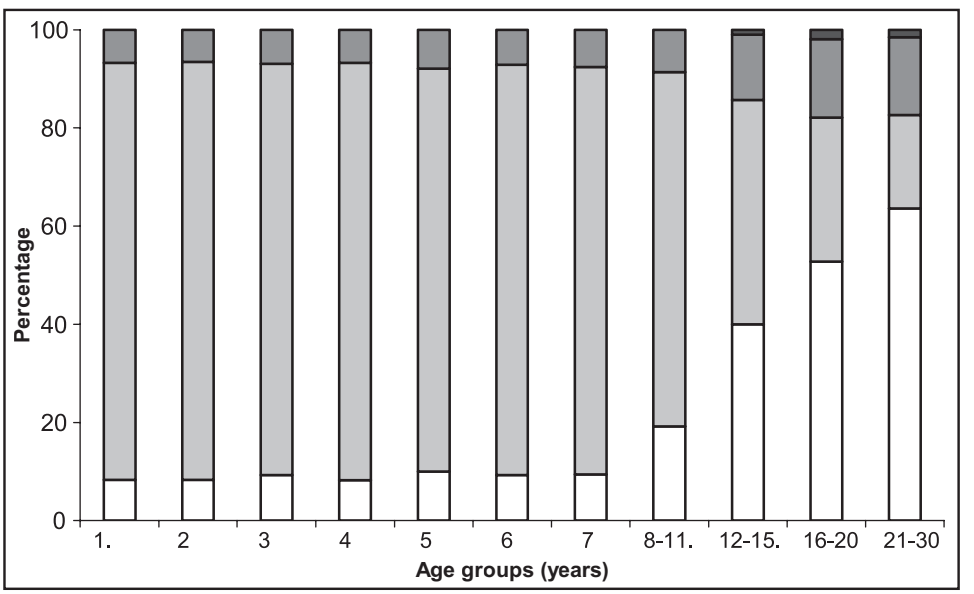

Fig. 1. Seroprevalence of HBV markers in the Turkish population in Northern Cyprus by age groups. ■, Anti-HBs-positive and anti-HBc-positive, natural infection; ■, HBsAg-positive, carriers; □, anti-HBs-positive and anti-HBc-negative, vaccinated; □, anti-HBs-negative, susceptible.

Table II. Prevalence of HBV Markers According to Sociodemographic Variables in the Turkish Population in Cyprus (n=585)

	Number	Anti-HBc	HBsAg	Anti-HBs
Age groups				
1-7	142	10 (7.0)*	0 (0)	128 (90.7)
8-11	105	9 (8.6)	0 (0)	88 (80.8)
12-15	105	14 (13.3)	1 (0.95)	63 (60)
16-20	106	19 (17.9)	2 (1.9)	50 (47.2)
21-30	132	23 (17.4)	2 (1.5)	48 (36.4)
p		0.044	ns**	<0.001
Gender				
Male	275	36 (13.1)	2 (0.7)	192 (69.8)
Female	310	41 (13.2)	3 (1)	218 (70.3)
p		ns	ns	ns
Location				
Urban	251	30 (12)	2 (0.8)	176 (70.1)
Rural	334	47 (14.1)	3 (0.9)	234 (70.1)
p		ns	ns	ns
Family size				
<5 people in house	309	46 (11.8)	4 (1)	271 (69.5)
≥5 people in house	176	30 (17)	1 (0.6)	125 (71)
p		ns	ns	ns
Parental education level				
<8 years				
≥9 years	117	20 (17.1)	2 (1.7)	90 (76.9)
	230	15 (6.5)	0 (0)	203 (88.3)
		0.002	0.047	0.006
Occupation				
Students	250	28 (11.2)	2 (0.8)	222 (88.8)
State employees	47	13 (27.7)	0 (0)	34 (72.3)
Industrial workers	41	6 (14.6)	1 (2.4)	29 (70.7)
Housewives	41	4 (9.8)	0 (0)	30 (73.2)
Health-care worker	20	3 (15)	0 (0)	17 (85)
Unemployed	8	4 (50)	1 (12.5)	5 (62.5)
Farmers	5	2 (40)	1 (20)	3 (60)
p		0.003	0.041	<0.001

*Numbers in parentheses, percent.
**Not significant.

The education level of parents was significantly correlated with HBV prevalence. Anti-HBc and HBsAg positivity rates were significantly higher in children of parents with low educational level (17.1% and 1.7%, respectively) compared to children of parents with higher educational level (6.5% and 0%, respectively) (Table II). Anti-HBc seroprevalence was slightly higher in large families with five and more members (17.0%) than in small families with four or fewer members (11.8%), but the difference was not statistically significant. Gender did not affect the seropositivity rates. There was no difference in seropositivity rates between rural and urban areas.

Seroprevalence of anti-HBc was higher in unemployed persons (50%) and farmers (40%) than in housewives (9.8%) and students (11.2%). Seropositivity of HBsAg was also higher in unemployed persons (12.5%) and farmers (20%). Health care workers had an infection rate of 15% and a vaccination rate of 80%. No health care workers in the study group had HBsAg (Table II).

Multivariant analysis revealed age and low parental educational level as the only independent risk factors for anti-HBc antibody positivity (Table III).

Discussion

There have been only two serological studies in Cyprus prior to this study. The first was performed in the Greek population of Cyprus in 1988 among blood donors, soldiers and risk groups, and HBsAg positivity rates were found

Table III. Odds Ratios (OR) for Associations Between Different Variables and Anti-HBc Antibody Positivity

	n	Odds ratio	95% CI
Age			
1-7	142	Reference	
8-11	105	0.99	0.39-2.42
12-15	105	1.77	0.81-4.19
16-20	106	2.37	1.02-5.53*
21-30	132	2.29	1.01-5.20*
Gender			
Male	275	Reference	
Female	310	1.01	0.62-1.63
Location			
Urban	251	Reference	
Rural	334	1.21	0.73-1.97
Family size	309		
<5 people in house		Reference	
≥5 people in house	176	1.53	0.93-2.53
Parental education			
<8 years	117	Reference	
≥9 years	230	2.95	1.45-6.01*

*Statistically significant.
CI: Confidence interval.

as 0.77% in blood donors and 1.01% in soldiers. The prevalence of past infection was between 11.1% and 13.6%[18]. This study reported that HBV infection should be considered as a major public health problem in Cyprus. The second study was performed in the Turkish population of Cyprus in 2006, and HBsAg positivity rates were found as 3.0% in blood donors and 2.7% in soldier candidates[19]. However, these studies were concentrated in selected population groups, like blood donors, army recruits, or high-risk groups, which are not representative of the general population. Thus, the present study is the first to be conducted with a systematic epidemiological approach on the prevalence of HBV infection in the Turkish population of Northern Cyprus.

In general, Cyprus is considered to be an intermediate endemic region[6]. At present, according to our findings, it seems that only 13.2% of the Turkish population under age 30 in Northern Cyprus was anti-HBc-positive. The proportion of HBsAg-positive persons in the population was 0.85%. Northern Cyprus thus complies with the criteria for a low endemic region (<2% HBV carriers and <20% HBV markers).

Hepatitis B virus prevalence in Northern Cyprus is markedly lower than reported from Turkey[5,20-22]. In a recent seroprevalence study, anti-HBc prevalence was found as 15.1% in the sera of 2,157 subjects under the age of 30 in Turkey[5]. According to the study results reported by Kanra et al.[5], HBsAg positivity was 5.4% in Turkey, whereas the positivity rate was found as only 0.85% in Northern Cyprus.

In Northern Cyprus, the universal hepatitis B infant immunization program was implemented in July 1998. We could not compare our results with prevaccination HBV infection rate, because prevaccination data are not available in Northern Cyprus. However, our study clearly shows the impact of the mass vaccination program started in 1998. In the 1-7 years of age group, anti-HBc seroprevalence was 7% and none of the children was HBsAg-positive. After 8 years of age, anti-HBc seroprevalence increased significantly with age, while vaccination rate decreased (Fig. 1). Hepatitis B infection rate was especially prominent in persons in the 16-20 years of age group, with a prevalence of 17.9%. Adolescents and young adults in the age group have an almost three-fold risk of HBV infection compared to the 1-7 years of age group ($p<0.001$), and 1.9% of them were HBsAg carriers. This population most likely came into contact with the infectious agent. On the other hand, the decrease in HBV prevalence in younger age groups was probably due to universal vaccination of all infants. The large majority of cohorts targeted

by the mass vaccination program possess protective antibody titers, while only 36% of young adults have protective antibody against hepatitis B. It is interesting to note that only 19% of adolescent and young adults have been vaccinated. Therefore, a catch-up immunization program targeted at adolescents and young adults in Northern Cyprus would seem to be useful to decrease HBV infection rate in the future.

The higher prevalence of hepatitis B markers in males as compared to females was reported in some studies[4,22,23]. However, in this study, the prevalence of HBV infection was found not different between males and females. On the other hand, working in health care facilities was not found as a risk factor for transmission of hepatitis B. Some studies also support that there is no significant seropositivity difference between health care workers and the other professions[4,21]. These results may be due to the higher vaccination rate of health care workers.

In this study, 2.9% of all samples were found to be positive for anti-HBc alone. Because of inadequate sample volumes, anti-HBc-positive but HBsAg- and anti-HBs-negative samples were not evaluated further for anti-HBc IgM and anti-HBe positivity. These persons may have acute HBV infection in the window phase, "low level" chronic HBV infection, past infection with anti HBs loss, or false-positive test results. The presence of anti-HBc alone varies depending on the population tested. In a German survey, 0.84% of the entire population was found to be positive for anti-HBc only. In another German study, however, this proportion was 19.2% in a high-risk group of convicts[24].

The results of our study show that the universal infant hepatitis B immunization program, which started in 1998 in Northern Cyprus, has had a substantial impact on immunity in children. However, prevalence of HBV infection is still high in adolescent and young adults. Therefore, a catch-up immunization program for these groups in addition to routine infant immunization will decrease the HBV infection rate and will help to reduce hepatitis B transmission in Northern Cyprus.

REFERENCES

1. World Health Organization. Hepatitis B vaccines. Wkly Epidemiol Rec 2004; 79: 255-263.
2. Shepard CW, Simard EP, Finelli R, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev 2006; 28: 12-25.
3. Mast E, Mahoney F, Kane M, Margolis H. Hepatitis B vaccine. In: Plotkin SA, Orenstein WA (eds). Vaccines (4th ed). Philadelphia: Saunders; 2004: 299-337.
4. Gogos CA, Fouka KP, Nikiforidis G, et al. Prevalence of hepatitis B and C virus infection in the general population and selected groups in South-Western Greece. Eur J Epidemiol 2003; 18: 551-557.
5. Kanra G, Tezcan S, Badur S, Turkish National Study Team. Hepatitis B and measles seroprevalence among Turkish children. Turk J Pediatr 2005; 47: 105-110.
6. Andre F. Hepatitis B epidemiology in Asia, the Middle East and Africa. Vaccine 2000; 18 (Suppl): 20-22.
7. World Health Organization. Expanded Programme on Immunization. Global Advisory Group - Part I. Weekly Epidemiological Record 1992; 67: 11-15.
8. World Health Organization. 2004 global immunization data. Geneva, Switzerland: World Health Organization, 2005. (http://www.who.int/immunization_monitoring/data/GlobalImmunizationData.pdf).
9. World Health Organization. WHO-UNICEF estimates of HepB3 coverage. Geneva, Switzerland: World Health Organization, 2005. (http://www.who.int/immunization_monitoring/en/globalsummary/timeseries/tswucoveragehepb3.htm).
10. Gilca V, Duval B, Boulianne N, Dion R, De Serres G. Impact of the Quebec school-based hepatitis B immunization program and potential benefit of the addition of an infant immunization program. Pediatr Infect Dis J 2006; 25: 372-374.
11. Tawk HM, Vickery K, Bisset L, Selby W, Cossart YE. Infection in Endoscopy Study Group. The impact of hepatitis B vaccination in a Western country: recall of vaccination and serological status in Australian adults. Vaccine 2006; 24: 1095-1106.
12. Shepard CW, Finelli L, Fiore AE, Bell BP. Epidemiology of hepatitis B and hepatitis B virus infection in United States children. Pediatr Infect Dis J 2005; 24: 755-760.
13. Viviani S, Mendy M, Jack AD, Hall AJ, Montesano R, Whittle HC. Hepatitis B vaccination in infancy in the Gambia: protection against carriage at 9 years of age. Vaccine 1999; 17: 2946-2950.
14. Mossong J, Putz L, Patiny S, Shneider F. Seroepidemiology of hepatitis A and hepatitis B virus in Luxembourg. Epidemiol Infect 2006; 134: 808-813.
15. Namgyal P. Impact of hepatitis B immunization, Europe and worldwide. J Hepatol 2003; 39 (Suppl): 77-82.
16. Rothenberg RB, Lobanov A, Singh KB, Stroh G Jr. Observations on the application of EPI cluster survey methods for estimating disease incidence. Bull WHO 1985; 63: 93-99.
17. Henderson RH, Sundaresan T. Cluster sampling to assess immunization coverage: a review of experience with a simplified sampling method. Bull WHO 1982; 60: 253-260.
18. Papaevangelou G, Roumeliotou A, Chatziminas M, et al. Epidemiological characteristics of hepatitis B virus infection in Cyprus. Eur J Epidemiol 1988; 4: 150-153.
19. Altındiş M, Yilmaz S, Dikengil T, Acemoglu H, Hosoglu S. Seroprevalence and genotyping of hepatitis B, hepatitis C and HIV among healthy population and Turkish soldiers in Northern Cyprus. World J Gastroenterol 2006; 14: 6792-6796.

20. Sidal M, Unuvar E, Oguz F, Cihan C, Onel D, Badur S. Age-specific seroepidemiology of hepatitis A, B, and E infections among children in Istanbul, Turkey. Eur J Epidemiol 2001; 17: 141-144.

21. Erden S, Buyukozturk S, Calangu S, Yılmaz G, Palanduz S, Badur S. A study of serological markers of hepatitis B and C viruses in Istanbul, Turkey. Med Princ Pract 2003; 12: 184-188.

22. Demirtürk T, Demirdal T, Toprak D, Altındıs M, Aktepe OC. Hepatitis B and C virus in West-Central Turkey: seroprevalence in healthy individuals admitted to a university hospital for routine health checks. Turk J Gastroenterol 2006; 17: 267-272.

23. Thierfelder W, Hellenbrand W, Meisel H, Schreier E, Dortschy R. Prevalence of markers for hepatitis A, B and C in the German population. Results of the German National Health Interview and Examination Survey 1998. Eur J Epidemiol 2001; 17: 429-435.

24. Neifer S, Molz B, Sucker U, Kreuzpaintner E, Weinberger, K, Jilg W. Hoher Prozentsatz isoliert anti-HBc positiver Personen unter Strafgefangenen. Gesundheitswesen 1997; 59: 409-412.