

Epidemiology, course and disease burden of chronic hepatitis B virus infection. HEPNET study for chronic hepatitis B: a multicentre Greek study

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SUMMARY. Hepatitis B virus infection (HBV) has been recognized as a major health problem worldwide. Greece belongs to the intermediate endemicity countries with a trend of decreasing prevalence of HBV infection during the last decade. However, the recent massive immigration to our country may have led to alterations of HBV epidemiology. In this study, we evaluated the epidemiological features of HBV infection in a sample of 3480 patients followed up during the years 1997–2006. Immigrants mainly from Albania represented the 18.6% of the total study population and 56.6% of children. The majority of the patients had no family history of HBV infection (67.3%) or of acute hepatitis (95.4%), no known source of infection (64.6%), with intra-familial spread accounting for 16.9% of the HBV transmission in adults and 33.9% in children. HBeAg(-) hepatitis B

was the predominant form of hepatitis (92.1%) among the Greek patients in contrast to the immigrants where 16.6% were HBeAg(+). Liver cirrhosis was diagnosed in 8.8% of the total population and 0.9% had hepatocellular carcinoma. A high proportion of children were HBeAg(+) (62%), 55% from immigrant families, 25.2% were infected in the perinatal period and had no evidence of disease complications. In conclusion our results showed (a) a changing pattern in the epidemiology of HBV infection in Greece due to the significant number of HBeAg(+) patients, especially among children and (b) a considerable number of patients although aware of their infection, present with advanced disease.

Keywords: epidemiology of hepatitis B, HBeAg(-) hepatitis B, HBeAg(+) hepatitis B, immigrants.

INTRODUCTION

Hepatitis B virus (HBVs) infection has been recognized as a worldwide health problem. It is estimated that more than two billion people alive today have been infected and 360 million of these suffer from chronic hepatitis B (CHB).

The prevalence of chronic HBsAg carriers in Europe ranges from <0.1% in north-western Europe to 1–8% in Southern Europe. In Southern Europe the prevalence of

chronic infection is intermediate and over half of CHB cases are related to infection with HBeAg-negative variants [1].

Greece belongs to the intermediate endemicity countries with a wide variance of seropositivity among different regions and various populations ranging from 3–5% [2] to 24.5% in certain parts of rural populations [3,4] and from 3.3% to 7.2% in certain high risk populations [5,6]. However, recent studies have reported a reduction of HBsAg prevalence ranging from 0.29% to 2.6% in the general population with male sex, old age and intrafamilial exposure being the major independent risk factors of HBV transmission [7–13]. This reduction is probably due to improvement of socioeconomic conditions and the implementation of vaccination programmes since 1998 when the Greek government integrated the hepatitis B vaccine in the existing

Abbreviations: CHB, chronic hepatitis B; CRF, case record form; HCC, hepatocellular carcinoma.

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childhood immunization schedules on a nationwide basis. Vaccination is now compulsory for newborns and for children entering puberty while high risk group immunization is also applied.

Nevertheless, demographic changes during the last decade resulting from the massive arrival of immigrants in the country and repatriated people mainly from Eastern Europe as well as immigrants from various countries with a high prevalence of HBV infection the last decade may lead to alterations in the HBV epidemic in our country. Indeed, epidemiological studies have reported a high HBsAg prevalence among immigrants from Albania (5.1–22.4%) [12,14,15] Eastern Turkey (7.1%) [16] and Asia (4.2–27.3%) [12,15].

HEPNET-GREECE for hepatitis B, is an ongoing nationwide retrospective–prospective study initiated in 1997. The study is sponsored by the Greek government, approved and conducted through the Hellenic Center for Disease Control and Prevention (HCDCP, KEELPNO, Greece). The main aims of the study are to evaluate the epidemiology and the course of HBV infection in Greece and their longitudinal changes.

Although patients enrolled in the HEPNET-GREECE study are still being followed to date, in this initial report we describe the patients with HBV infection baseline demographics, clinical and virological characteristics at their initial presentation at a hepatology centre. Furthermore, we evaluated those characteristics among immigrants and Greek patients.

PATIENTS AND METHODS

In 2003 the HEPNET-GREECE network was established in order to collect and evaluate data regarding patients with chronic viral hepatitis B and C in Greece. Nineteen hepatology centres throughout the country participate in the HEPNET-GREECE study. Patients were identified with positive HBsAg (a) through occasional screenings (blood bank, workplace, immigrants, etc.) (b) with elevated transaminases from routine health tests or (c) with clinical symptoms of liver disease and referred to one of the Hepatology Centres. All patients found to be HBsAg(+) were advised to inform their relatives in order to be checked. However, in this retrospective–prospective cohort study, detailed information on this procedure was not collected, therefore the secondary cases of intrafamilial exposures with HBV were not estimated. All HBsAg positive individuals followed in the above centres that met the following criteria were enrolled in the study. Inclusion criteria were: (1) HBsAg positivity for at least 6 months and (2) all patients who were under follow-up on 1 January 1997, regardless of treatment and final clinical outcome, or patients who initiated their follow-up at the participating centres during the period from 1 January 1997 until the end of June 2006. Patients coinfecting with HIV or HCV were excluded.

A structured case record form (CRF) was used for data collection. Data were prospectively updated twice a year. The method of CRF was replaced in 2004, by a modern, up-to-date electronic data. All CRFs were submitted to the statistical and data management centre of the study based in the Department of Hygiene and Epidemiology of Athens University Medical School. Data quality controls were performed for the detection of possible duplications, logical errors, missing values or unacceptable values and data queries were generated and sent to the respective Centre. After 2005 the data have been reported directly to a properly set-up electronic database.

Study entry was considered as the date of patients' first visit to the clinic or the date of first HBV diagnosis if that occurred following the patients first visit.

At study entry, detailed data on patients' self-reported demographic characteristics (gender, source of infection, BMI, birth place, residence place, occupation, educational level, marital status, number of children, smoking and alcohol drinking habits) were collected as well as clinical signs, other chronic diseases and treatment history. In relation to the source of infection, whenever multiple sources of infection were reported, doctors decided on the most likely one based on the information they gathered from the patients as well as on the recognized HBV epidemic characteristics. In this study, among several reported possible sources of infection, the intrafamilial was considered the most likely one followed by blood transfusion in cases of infection before 1975.

Ethnicity as well as the terms of urban, semi-urban or rural were defined based on the patients place of birth.

Biochemical, virological, histological, serological and ultrasounds' findings were also recorded. HBV-DNA was not measured in a central lab, as in most population-based multicentre epidemiological studies. For the HBV-DNA determination, the PCR method (qualitative assay in 29.6%, quantitative assay 70.4%) was used in 77.5% ($n = 1574$) of the patients (the Cobas-Amplicor in 21.3%, $n = 433$), Genostics (qualitative 59.6%, quantitative 40.4%) in 14.4% ($n = 292$) and other assays (qualitative 48.8%, quantitative 51.2%) in 8.1% ($n = 166$).

Diagnosis of cirrhosis was based on histological findings, provided that liver biopsies were conducted within 6 months from study entry. In the absence of a baseline liver biopsy, a classification was made based on clinical (ascites or flapping tremor), biochemical (synthetic capacity of the liver) and imaging (nodules in the hepatic parenchyma, splenomegaly, diameter of portal venous >16 mm) data at study entry.

Statistical analysis

Descriptive statistical analysis was performed for baseline patients' characteristics at the first visit. Results were expressed as absolute and relative frequencies for qualitative variables and as medians and interquartile ranges for

Table 1 Demographic characteristics of the study population by age category and ethnicity

	Adults	Children	P-value [†]	Greeks	Immigrants	P-value [‡]	Total
Gender, males (n, %)	2135	78	0.604	1655	386	0.492	2213
Birth place (n, %)			<0.001			<0.001	
Urban	1265	7		1181	87		1272
Semi-urban	369	17		318	66		386
Rural	1053	49		886	215		1102
Age at first visit (years) (median, IQR [§])	46.2	8.2	<0.001	48.4	29.2	<0.001	45.2
	33.7–56.4	4.6–11.4		36.9–57.8	20.8–38.5		32.2–56.0
BMI (kg/m ²) category (n, %)			<0.001			<0.001	
≤25	975	65		727	300		1040
25–30	1048	10		886	150		1058
>30	342	1		302	29		343
Family history (n, %)	1047	74	<0.001	894	181	0.662	1121
History of acute infection (n, %)	152	3	0.262	111	34	0.110	155
	4.6	2.5		4.3	6.0		4.5
Source of infection (n, %)			<0.001			0.136	
Perinatal	248	32		223	53		280
Intrafamilial	567	43		493	100		610
Transfusion	53	1		47	4		54
Other	318	1		259	52		319
Unknown	2167	50		1612	391		2217
	64.6	39.4		61.2	65.2		63.7

[†]Comparison by age category. [‡]Comparison by ethnicity. [§]Interquartile range.

continuous variables. Comparisons of categorical characteristics by age category and ethnicity were carried out using the chi-square test or the Fisher's exact test as appropriate. *P*-values less than 0.05 were considered as statistically significant. The total number of patients shown in the different tables within the article may differ due to missing data in specific covariates.

RESULTS

In total 3762 patients with HBV infection were identified, whereas 3480 fulfilled the inclusion/exclusion criteria and were included in the present analysis. The study population consisted of 3353 (93.4%) adults, i.e. >14 years old at first visit and 127 (3.6%) children. Ethnicity was known for 3234 patients (92.9%). The majority of non-Greeks were born in Albania (71.0%) followed by Eastern Europe (19.4%) and Asia (3.2%). For this analysis Greeks and those born in the Western Europe were classified as Greeks, whereas the remaining of the study population were classified as immigrants.

The baseline demographic characteristics by ethnicity and age group are shown in Table 1. The majority of the patients were male, with an average age of 45 years old, born in urban areas and more than half of them were overweighted. There was a high percentage of unknown source of infection, however among those with known route of infection the most frequently reported source was intrafamilial spread. Children were more frequently born in rural areas, had lower BMI, higher percentage of family history and mostly known source of infection compared to adults. Immigrants were more frequently born in rural areas, were younger and with lower BMI compared to Greeks. No difference based on both by age and ethnicity was observed for gender and history of acute infection. Limiting the analysis to the adult

population with smoking habits information, 820/3048 (26.9%) were current smokers, 28.1% and 24.4% for Greeks and immigrants, respectively. Regarding the educational level, 68/2656 (2.6%) were illiterate and 526/2656 (19.8%) had higher education.

Clinical signs of advanced liver disease at presentation such as hepatomegaly, splenomegaly, ascites, flapping tremor and icterus were observed at 18.1%, 8.6%, 2.6%, 0.8% and 1.6%, respectively. The stage of disease based on histological evaluation, was available for 36.2% (*n* = 1260) of the study population, and for 9.8% (*n* = 124) of those, cirrhosis was diagnosed. One hundred and seventy six additional patients from those without a baseline biopsy, were classified as cirrhotics based on the clinical and ultrasound evaluation, resulting in a total of 300 (8.8%) patients with cirrhosis at study entry. Classification was not feasible

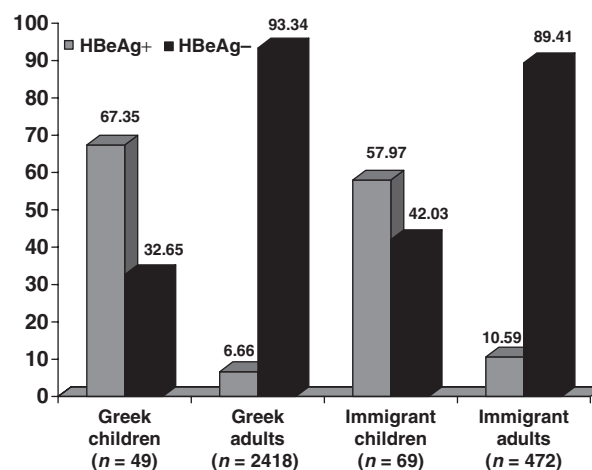


Fig. 1 Distribution of patients according to age, ethnicity and HBeAg status.

Table 2 Characteristics of the study population by age category and ethnicity[†]

	Adults		Children		<i>P</i> -value [‡]	Greeks		Immigrants		<i>P</i> -value [§]	Total	
Liver cirrhosis (<i>n</i> , %)	299	9.1	1	0.8	0.001	257	10.1	24	4.6	<0.001	300	8.8
ALT levels, high [¶] (<i>n</i> , %)	1264	49.5	62	65.3	0.003	977	50.1	193	44.0	0.020	1326	50.1
HBV-DNA, detectable ^{**} (<i>n</i> , %)	1555	74.1	67	81.7	0.119	1216	72.9	228	80.6	0.006	1622	74.3
BIL, ≥1.1 mg/dL (<i>n</i> , %)	442	21.8	4	10.8	0.109	346	22.0	71	20.2	0.460	446	21.6
Platelet count, <150 000/mm ³ (<i>n</i> , %)	438	20.6	1	1.8	0.001	361	22.1	47	12.4	<0.001	439	20.1
History of treatment ^{††} (<i>n</i> , %)	895	26.7	36	28.3	0.679	28.3	28.3	85	16.0	<0.001	931	26.8
Ongoing treatment ^{‡‡} (<i>n</i> , %)	157	4.7	0	0	0.004	124	4.8	17	3.2	0.106	157	4.51

[†]Comparisons by ethnicity restricted to the adult population. [‡]Comparison by age category. [§]Comparison by ethnicity. [¶]Higher than the upper normal limit used (40 IU/L). ^{**}Based on qualitative (37%) or quantitative methods. ^{††}Treatment received before study entry or initiated at study entry. ^{‡‡}Under treatment at study entry (initiated before or at study entry).

for 84 patients. One hundred and fifty four (4.4%) patients came with advanced disease at study entry. In particular, 125 had decompensated cirrhosis and 29 HCC. Comparison of patients' clinical characteristics by age category and country of birth is presented in Table 2. Overall, more than half patients presented with high ALT values and detectable HBV-DNA. Greek patients had significantly higher levels of ALT ($P = 0.02$). Among children, 60% of the immigrants and 73% of the Greeks had elevated ALT levels, however the difference was not statistically significant. A considerable proportion of patients (26.8%), had a history of treatment until the study entry with 4.5% being on treatment at study entry. Children were significantly less cirrhotics than adults and none of them was on treatment. Adult immigrants were less likely to have developed cirrhosis and to have a low platelet count at study entry, but they presented more frequently with detectable HBV-DNA compared to Greek adults. Treatment history as well as ongoing treatment was more common in the Greek adult population.

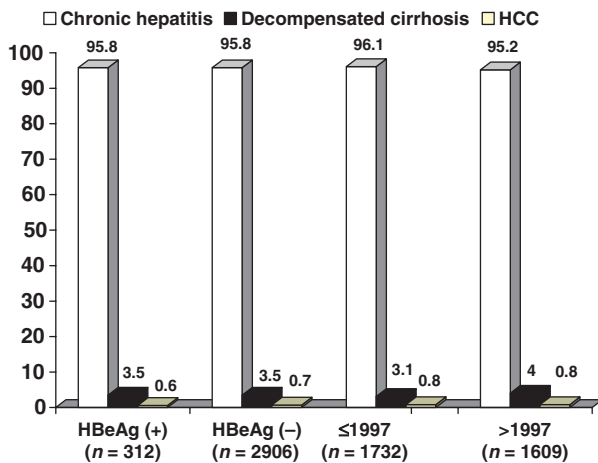


Fig. 2 Clinical status in correlation to HBeAg and year of first HBV diagnosis.

Table 3 Characteristics of the study population by HBeAg status

	HBeAg(+) (N = 313)	HBeAg(-) (N = 2918)	P-value
Age, years, median (IQR) [†]	23.6 (14.5–39.6)	46.7 (34.6–56.6)	<0.001
Gender, female (n, %)	109 (34.8)	1072 (36.7)	0.504
Liver cirrhosis (n, %)	15 (4.8)	251 (8.8)	<0.017
ALT, high [‡] (n, %)	182 (78.8)	1067 (47.5)	<0.001
HBV-DNA, detectable (n, %)	220 (92.1)	1329 (71.7)	<0.001
BIL, ≥1.1 mg/dL (n, %)	42 (27.3)	377 (21.1)	0.073
Platelet count, <150 000/mm ³ (n, %)	33 (18.0)	367 (19.8)	0.574
History of treatment (n, %)	140 (44.7)	743 (25.5)	<0.001
Ongoing treatment (n, %)	4.79 (4.8)	121 (4.1)	0.589

[†]Interquartile range. [‡]Higher than the upper normal limit used or than 40 IU/L if the upper normal limit was missing.

Data regarding the HBeAg status was available for 3231 patients. In total, the great majority (90.3%) of the study population was HBeAg(-). Highly significant differences in HBeAg distribution were observed between adults and children (HBeAg(+): 7.6% vs 62.3%, respectively; $P < 0.001$) and between immigrants and Greeks (HBeAg(+): 16.6% vs 7.9%, respectively; $P < 0.001$). Figure 1 presents the composition of HBeAg categories based on adults and children by ethnicity. An increased percentage of children was observed in the subpopulation of HBeAg positive immigrants compared to positive Greeks (44.4% vs 17%). In addition, adult immigrants had more frequently positive HBeAg compared to the Greek adults (10.6% vs 6.7%, respectively; $P = 0.003$). However, this difference was not observed among women of reproductive age (9.3% vs 9.0%, $P = 0.947$) but the number of women in our study was small (42 Greek women and 14 immigrants). Therefore, the overall increased HBeAg(+) prevalence in the immigrants reflects the overrepresentation of positive children, but to some extent this can also be explained through the higher positivity in the immigrants adult population. Figure 2 presents the clinical status of the patients in correlation to HBeAg and the year of first HBV diagnosis. The year of 1997 was chosen as it represents the median year of first HBV diagnosis of the study group.

Differences in patients' characteristics according to the HBeAg status were further investigated and are presented in Table 3. Positive patients were significantly younger and although presented less frequently with cirrhosis they had higher ALT and HBV-DNA values. Restricting the analysis to the adult patients, no significant difference by HBeAg categories was observed with respect to baseline cirrhosis.

DISCUSSION

Although an effective vaccine against hepatitis B has been available since 1982 and WHO recommended that hepatitis B

vaccine should be included in routine immunization schedules in all countries, the disease burden due to HBV infection remains undiminished in many parts of the world [17]. The prevalence of chronic HBV infection varies geographically and new infections occur in people who have emigrated from high prevalence countries [18,19].

Furthermore, for the foreseeable future, patients already chronically infected with HBV will experience the major complications of chronic infection, including decompensated cirrhosis and hepatocellular carcinoma (HCC) [20, 21].

Greece has been associated with intermediate endemicity countries with a wide variance of seropositivity among different regions and various populations [3–6]. However, recent studies reported a reduction of HBsAg prevalence ranging from 0.29% to 2.5% due to improvement of socio-economic conditions and the implementation of hepatitis B vaccination in routine immunization schedules since 1998. Nevertheless, global distribution of HBV is being affected by population movements from high prevalence areas. Indeed during the last decade, immigrants from countries with an increased prevalence of HBV infection have settled in Greece. Thus, demographic changes resulting from this massive influx of immigrants, may lead to alterations of HBV disease prevalence in our country.

In this retrospective study we evaluated hepatitis B infection in a large cohort of 3480 patients in total during 1997–2006. We found that there were differences between Greeks and immigrants regarding their HBeAg positivity with refugees being more frequently HBeAg(+) than Greeks.

The results of the present study showed that immigrants consisted of a considerable proportion (18.6% of the total study population) and 56.6% of the children. Patients from Albania were found to be the core immigrant population (13.7% out of 18.6%). Previous epidemiological studies have shown a high prevalence of HBV infection among immigrants from Albania registered in Greece [12,14,15] and have suggested that adherence to general precautions and an effort to improve their living conditions as well as initiation of HBV vaccination programmes will be necessary in these communities. Since WHO reports that hepatitis B vaccine has been implemented in infant vaccination programmes in Albania since 1996 with a coverage rate of 88% [18], these findings of high HBV prevalence rates among immigrants from Albania could partially be explained by the fact that the main population movement to Greece from Albania occurred earlier in the decade of 1990s. In any case, however, these findings of high HBV prevalence are expected to possibly affect the epidemiology pattern of HBV infection in our country.

Immigrants were of younger age both at first visit (30.3 ± 14.2) and at first diagnosis of HBV infection (28.4 ± 13.7). Most of them discovered their HBV infection during HBsAg screening of blood donors. Another interesting finding was that many of the patients enrolled in the present study, although aware of their disease before their

first visit to the centre, did not seek medical advice, probably due to ignorance about the seriousness of their disease.

It should be mentioned that legalized immigrants have in principle the same access to medical care as Greek patients. Regarding not legalized immigrants, there is a recent legal circular in use that gives them the possibility to receive the appropriate treatment free of charge if they suffer from an infectious disease. However, in practice things may not work out as hypothesized as immigrants (both legalized and not) is likely to feel afraid or uncomfortable in seeking medical care either because of language barriers or due to their general insecure status.

Regarding the possible source of infection, most of the adult population, either natives or immigrants, had no known source of infection with intrafamilial spread accounting for 16.7% and 18.7% of the HBV transmission, respectively. These results are in agreement with data from areas with intermediate HBV infection including the Mediterranean countries and Eastern Europe, where household, sexual and perinatal are the main routes of HBV transmission [22].

In recent studies from different parts of the world, the prevalence of HBeAg negativity in chronic HBV infection has been found to range from 70% to 100% [23–25]. In the Mediterranean area, most cases are related to Hepatitis B 'e' variants [22]. Furthermore, there is evidence of an increasing trend towards HBeAg-negative HBV infection in many areas of the world [26–29] with HBeAg-negative hepatitis B being the predominant form in hepatitis in Europe (60–90%) [25]. The results of the present study have confirmed that HBeAg-negative hepatitis B is the predominant form of hepatitis B in Greece, accounting for 92.13% of the cases among the native adult population. An interesting finding however, possibly having a serious impact on the epidemiology of HBV infection in Greece, was that immigrants showed a significant higher proportion of hepatitis B related to HBeAg(+) HBV infection (16.6%) with significantly lower ALT and significantly higher HBV-DNA levels.

As far as disease progression is concerned, it was found that 8.8% of the study population had already cirrhosis at study entry and approximately 5% presented with advanced stage, specifically decompensated cirrhosis and HCC. As a consequence, this result causes special public health implications especially regarding early diagnosis and screening interventions. These findings are in agreement with data showing that at presentation most patients with either HBeAg(+) or HBeAg(-) chronic hepatitis B are asymptomatic having been discovered incidentally during HBsAg screening of blood donors or evaluation of asymptomatic ALT elevations or some patients becoming aware of their underlying liver disease only as it reaches an advanced stage with complications [25].

Chronic hepatitis B in childhood appears to present some unique features depending on age at primary HBV infection and route of transmission [30,31]. Transmission can be

either perinatal or horizontal. It is reported that in the Mediterranean area where the proportion of HBeAg positive mothers is much lower, route of transmission is mainly horizontal occurring during infancy or childhood [29]. In the present study, quite a high proportion of children – up to 25.2% – were infected during the perinatal period, in 33.9% transmission of HBV was horizontal by HBsAg positive family members and in 39.4% the source of infection was unknown. It is rather difficult to interpret the perinatal infection of the children due to the fact that most mothers would be HBeAg(–) according to the epidemiological data in our country. However, taking into account that a significant proportion of the children were HBeAg(+) (62.3%) and that 55% were from immigrant families with a higher proportion of HBeAg(+) positivity, some of these children could have been born to HBeAg(+) mothers.

It has been reported that many Asian children who were infected in the perinatal period and developed chronic HBeAg(+) hepatitis B, usually have normal ALT and minimal liver damage (31). Our findings are in agreement with these data since all of the children in the present study, presented with CHB and no evidence of decompensated cirrhosis or HCC.

There are, however, some limitations concerning our results: (1) the retrospective design of the study may lead to selection bias. This limitation is minimized due to (i) the large number of participating centres and patients, (ii) the quality control of the data and (iii) the prospective design of the study after 2004. (2) The population of participating clinics may not be representative of Greek HBV patients. However, observed trends of our study reflect the general trends in Greece. (3) Provided that ethnicity was defined based on place of birth, it is likely that the immigrants' contribution to chronic HBV has been somewhat underestimated. However, the degree of underestimation could be limited due to the fact that massive immigration only happened in late 1990's, especially from Albania.

Hepatitis B infection is of major global importance because of the large pool of patients, the serious nature of the disease sequelae, the difficulty to eradicate once chronic infection is established and the significant economic burden. The results of the present study have shown a changing epidemiology of HBV infection in our country due to the significant number of immigrants registered in Greece the last decade, with a significant number of patients presenting with HBeAg(+) hepatitis B and even more with a high proportion of HBeAg(+) children. Furthermore, it has been shown that a considerable number of patients although aware of their infection do not seek medical assistance immediately, hence their disease is already advanced in a number of them. In that scope, urgent actions are needed to address the problem: immunization schedules of the immigrant families and education initiatives to prevent transmission of the hepatitis B virus. Free of charge screening for HBV infection should be an option for immigrants in Greece, on the presumption that

the process is not obligatory and the results of the test will not be used for purposes other than medical care and prevention. Public campaigns should regularly and continuously be implemented, not only in Greek language but also in most frequently spoken languages among immigrants, in order to sensitize people regarding HBV transmission modes and prevention measures.

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