Hepat Mon. 2017 August; 17(8):e13260. doi: 10.5812/hepatmon.13260.

Published online 2017 May 30. **Research Article**

Hepatitis B Virus Genotypes, Epidemiological Characteristics, and Clinical Presentation of HBV Chronic Infection in Immigrant Populations Living in Southern Italy

Evangelista Sagnelli,[1,*] Loredana Alessio,[2] Caterina Sagnelli,[1,3] Luciano Gualdieri,[4] Mariantonietta Pisaturo,[5,6] Carmine Minichini,[1] Giovanni Di Caprio,[1,2] Mario Starace,[1] Lorenzo Onorato,[1,2] Margherita Macera,[1] Gaetano Scotto,[7] and Nicola Coppola[1]

[1]Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, Italy
[2]Medical Center, Centro Sociale ex Canapificio, Caserta
[3]Medical Center, Centro Suore Missionarie Della Carita, Naples, Italy
[4]Medical Center, Centro per la Tutela Della Salute Degli Immigrati, Naples, Italy
[5]Medical Center, Centro di Accoglienza "La tenda di Abramo", Caserta, Italy
[6]Infectious Diseases Unit, AORN Sant'Anna e San Sebastiano, Caserta, Italy
[7]Infectious Diseases Unit, Foggia

[*]*Corresponding author*: Professor Evangelista Sagnelli, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, Via: L. Armanni 5, 80131 Naples, Italy. Tel: +39-815666719, Fax: +39-815666207, E-mail: evangelistasagnelli@libero.it

Received 2016 October 22; **Revised** 2017 February 12; **Accepted** 2017 March 07.

Abstract

Background: Information on hepatitis B virus (HBV) genotypes in immigrant populations living in Italy is scanty.
Objectives: The current study aimed at assessing the epidemiological and clinical need to detect HBV genotypes in immigrants with HBV infection.
Methods: A multicenter screening was performed in 5 first-level care facilities centers in Southern Italy to identify migrants with HBV infection. Hepatitis B surface antigen (HBsAg)-positive subjects were further investigated at a tertiary unit of infectious diseases.
Results: Of the 1727 investigated immigrants, 170 (9.8%) were HBsAg-positive. These 170 subjects, prevalently males (86.5%), aged 31.0 ± 8.5 years and living in Italy for nearly 2.5 years, prevalently (80%) from sub-Saharan Africa. HBV DNA was detected in 113 (66.5%) and HBV genotypes in 109 subjects: genotype E in 69.9%, genotype A in 16.5%, genotype D in 11.9%, and genotype C in 2.7%. Genotype E was detected in 70 (83.3%) out of 84 migrants from sub-Saharan Africa and in 5 from the other areas. Of these 75, 16% were hepatitis B e antigen (HBeAg)-positive and none circulated anti-hepatitis D virus (HDV); 69.3% were inactive HBV carriers, 22.7% had chronic hepatitis and 8% cirrhosis with multifocal hepatocellular carcinoma (HCC) in 2 patients. Half of the 18 subjects with genotype A, prevalently from sub-Saharan Africa (61%), were inactive HBV carriers, 7 had chronic hepatitis, and 1 had liver cirrhosis. Of the 13 subjects with genotype D, prevalently from Eastern Europe or India-Pakistan subcontinent, 8 were HBV inactive carriers and 5 had chronic hepatitis.
Conclusions: The data indicated the need to extend HBV screening and vaccination programs to all immigrants from areas of intermediate or high HBV endemicity.

Keywords: HBV Infection, Chronic Hepatitis B, HBV in Immigrants, Liver Cirrhosis

1. Background

Although using a reverse transcriptase lacks proofreading activity for the replication, hepatitis B virus (HBV) presents high genetic variability expressed by the 10 genotypes alphabetically identified from A to J and divided into sub-genotypes. HBV genotypes and sub-genotypes have a distinct ethno-geographical distribution (1), consequent to HBV co-expansions with ancient and modern human migrations (2). Genotype A predominates in Northern-Western Europe, North America, and Eastern Africa (3), genotype D in the Mediterranean area (4-12), Middle-East and Southern Asia (13, 14), genotypes B and C in Asia (15), genotype E in Central-western Africa (13), genotype F in South and Central America (13), genotype G in France and the United States (15), genotype H in the Northern part of Latin America (16) and genotypes I and J in Eastern Asia (1, 17).

The global distribution of HBV infection varies significantly between countries and between the regions within a country. Western Europe, the USA, Canada, and some South American and Northern African countries present a low level of endemicity with a rate of hepatitis B surface anti-

gen (HBsAg) chronic carriers below 2%, whereas this prevalence is intermediate in Eastern Europe, Central Asia, and some East Asian countries (from 2% to 8%) and high in some Asian and sub-Saharan countries and Alaska (above 8%), sustained by perinatal and childhood transmission (18, 19).

Due to the socioeconomic and political crises in Africa, Eastern Europe, and Central and Eastern Asia in recent decades, Western countries are the lands of immigration from geographical areas of intermediate or high HBV endemicity (20). In 2014, 4,922,085 legal immigrants were living in Italy, mainly from Eastern Europe (52.3%), Africa (22.6%), Asia (16%), and South America (6.3%). It is also estimated that nearly 500,000 undocumented immigrants currently live in Italy (21).

As an effect of immigration, the introduction and spread of new HBV genotypes are documented in patients with acute hepatitis (4-6, 8, 11, 12, 22-25), whereas information on HBV genotypes in undocumented and refugee immigrants with chronic HBV infection living in Italy is scanty (7, 9, 13, 26, 27). To improve the knowledge on this topic, the current prospective study was conducted to evaluate the epidemiological and clinical impact of HBV genotypes in 170 undocumented or refugee immigrants with chronic HBV infection, prevalently born in sub-Saharan Africa, Eastern Europe, or the India-Pakistan subcontinent and permanently living in Naples (Italy) or suburban areas.

2. Objectives

The current study aimed at determining the HBV genotypes in a cohort of 170 undocumented or refugee immigrants with chronic HBV infection identified in a screening program conducted on 1727 immigrants.

3. Methods

3.1. Study Population

It was a multicenter, prospective study with the participation of 5 first-level care facilities centers in Southern Italy, 4 in the Campania region (2 in Naples and 2 in Caserta) and 1 in the Apulia region (in Mezzanone, near Foggia), and 3 tertiary care units for infectious diseases, 1 in Naples, 2 in Caserta, and 1 in Foggia. The urban and suburban areas of Naples, Caserta, and Foggia give medical services to a large population of refugees and undocumented immigrants from Africa, Asia, Eastern Europe, and Latin America. Preliminary information on this screening was reported in a previous paper (26). The screening program was started from January 2012 covering all undocumented immigrants and refugees consecutively referred for a clinical consultation at 1 of the 5 first-level care facilities centers up to December 2015. In Italy, the juridical definition of refugees refers to immigrants who have fled their countries of origin because of war or political, religious, or racial discrimination, whereas undocumented immigrants are the ones who have come without any legal permit in search of work or better living conditions. The refugees have access to all the healthcare facilities of the Italian national healthcare system, whereas for undocumented immigrants this access is limited to minors, pregnant females and patients with serious pathological conditions or transmissible diseases. Because of the reduced economic resources in the last decade, the undocumented and refugee immigrant populations generally have poor living conditions in Italy, with a low income most frequently from casual day-to-day work. In addition, they are prevalently young, without family ties, and not integrated due to language, cultural, and social barriers.

The first-level care facilities centers participating in the present study are the outpatient clinics of general medicine with a long-term experience in the management of vulnerable groups, consulted by refugees and undocumented immigrants mostly for lumbago, headache, pruritus, cough, high blood pressure, and allergy symptoms. During a clinical consultation, a physician and a cultural mediator explained to the immigrants the importance of testing for HBV, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) serum markers and offered them screening free of charge, in anonymity, and in full compliance with the Italian privacy law. During the study period, adhesion to the screening and a signed informed consent, written in the immigrant's native language, was obtained on a voluntary basis from 85% of the participants (1,727 out of 2,032). Information on the demographics, socioeconomic status, clinical data, and risk factors for acquiring HBV, HCV, and HIV infections were collected in an anonymous questionnaire for 1727 subjects who agreed to participate in this investigation.

The serum sample obtained at the time of the clinical consultation was tested for HBsAg, HCV-antibody (Ab) and HIV-Ab.

No inclusion or exclusion criteria were applied. All HBsAg-positive refugees and undocumented immigrants were referred to the nearest tertiary care unit of infectious diseases for further investigation, monitoring, and possible treatment. These units have cooperated for over 15 years in several clinical investigations, using the same clinical approach and the same laboratory methods (13, 27, 28). The cultural mediators assisted the HBsAg-positive subjects both at the first-level and third-level care facilities units.

The current paper reports the demographic, epidemiological, and clinical characteristics of 170 HBsAg-positive

subjects (9.8%) out of the 1727 screened ones. The diagnosis of HBV inactive chronic carriage was made based on the persistence of normal alanine aminotransferase (ALT) serum values for 6 months or more, HBV DNA absence or below 2000 IU/mL, and the absence of clinical, biochemical, and ultrasound evidence of liver cirrhosis. The diagnosis of HBV-related chronic hepatitis was based on the persistence of ALT abnormality for 6 months or more in subjects who did not show clinical, biochemical, or ultrasound evidence of liver cirrhosis (29). HBV-related liver cirrhosis was diagnosed on the basis of the clinical, biochemical, and ultrasound signs (11). According to the accepted criteria, HBV-related hepatocellular carcinoma (HCC) was diagnosed on the basis of histological and/or imaging findings and on α-fetoprotein (AFP) serum levels (30).

The current study was approved by the Ethics Committee of Azienda Ospedaliera Universitaria of Second University of Naples (protocol number 214/2012)

3.2. Serum Markers of HBV, HCV, and HIV Infections

Serum samples were tested for HBsAg, anti-HCV, anti-HIV, total hepatitis B core antigen (anti-HBc), and hepatitis B surface antibody (anti-HBs) by commercial immunoenzymatic assays (Abbott Laboratories, NorthChicago, IL, USA): AxSYM HBsAg (V.2) M/S for HBsAg, AXSYM HCV 3.0 for anti-HCV, AXSYM HIV 1/2 COMBO for HIV, AXSYM core for total anti-HBc and AXSYM AUSAB for anti-HBs). Anti-HIV reactivity was always confirmed by a Western blot assay (Genelabs Diagnostics, Science Park Drive, Singapore), which identifies both HIV1/2 strains.

3.3. HBV-DNA Determination by PCR

HBV-DNA serum levels were determined by a real-time PCR with a detection limit of 20 copies/mL as previously described (27). HBV genotypes were determined in HBV-DNA-positive serum samples (31). Specifically, HBV genotypes were determined by the phylogenetic analysis of sequences of 500 nt of the S region by a nested PCR, using in the first PCR the outer primers F-2862 forward 5'-TCACCATATTCTTGGGAAC-3' and R-853 5'-AGGGTTTAAATGTATAACCCA-3', and in the second PCR the inner primers F-179 5'-CTAGGACCCCTGCTCGTGTT-3' and R-690 reverse 5'-AATGGCACTAGTAAACTGAG -3'. The PCR products were, then, purified using the MinElute PCR Purification Kit (Qiagen, Qiagen GmbH); the sequencing reaction was performed using the forward primer F-179 5'-CTAGGACCCCTGCTCGTGTT-3' and reverse R-690 5'-AATGGCACTAGTAAACTGAG-3' with the Big Dye™ terminator v 1.1 cycle sequencing kit (applied biosystems, Carlsbad, CA, USA) in an ABI 310 genetic analyzer (applied biosystems). Sequences were aligned using the BioEdit program with reference sequences for the different genotypes deposited in GenBank (accession numbers: X02763, X51970, and AF090842 for genotype A; D00329, AF100309, and AB033554 for genotype B; X04615, M12906, and AB014381 for genotype C; X65259, M32138, and X85254 for genotype D; X75657 and AB032431 for genotype E; X69798, AB036910, and AF223965 for genotype F; AF160501, AB064310, and AF405706 for genotype G; and AY090454, AY090457, and AY090460 for genotype H). Genetic distances were calculated with the Kimura-2-parameter algorithm and a neighbor-joining phylogenetic tree was constructed with the Mega 6 program http://www.megasoftware.net (center of evolutionary functional genomics, biodesign institute, Arizona State University, USA).

3.4. Statistical Analysis

Continuous variables were summarized as mean and standard deviation (SD) and categorical variables as absolute and relative frequencies. Differences in the mean values were evaluated by the Student t test, and the Chi-square test was applied to categorical variables. A P value < 0.05 was considered statistically significant.

4. Results

From January 2012 to December 2015, a total of 1727 immigrants were screened, and 170 (9.8%) of them were HBsAg-positive. The characteristics of these 170 are shown in Table 1. They lived in Italy for a mean period of 32.4 ± 8.5 months, were prevalently male (86.5%), with a mean age of 31.1 ± 8.5 years and prevalently undocumented (65.3%). Of these 170 subjects, 80% came from sub-Saharan Africa, 10% from Eastern Europe, nearly 8% from the India-Pakistan subcontinent, almost 2% from Northern Africa and only 1 subject from South America. They had a low level of schooling (mean 6.3 ± 4.2 years) and only a few of them stated alcohol consumption, occasional in most cases (Table 1). Almost all stated the risk factors for the inapparent parenteral exposure, including unsafe therapy injection, acupuncture, tattoo, piercing, and tribal practices, about 43% invasive medical procedures including surgery, dental care, and abortion, and nearly 24% unsafe sexual activity. No subject stated drug addiction and only 2 had received a blood transfusion.

Of the 170 HBsAg-positive subjects identified in this screening, 133 (78.2%) had an inactive chronic liver disease (CLD), 29 (17.1%) chronic hepatitis, and 8 (4.7%) liver cirrhosis. All 170 subjects were tested for HBV DNA and HBV genotyping at 1 of the 3 participating tertiary care units.

HBV DNA was detected in 113 (66.5%) subjects and was undetectable in 57 (33.5%). Of the 113 HBV-DNA-positive sub-

Table 1. Demographics and Epidemiological Characteristics of the Studied Immigrants with HBsAg-Positive and Chronic Liver Disease (N = 170)

Variables	Value
Age, y (Mean ± SD)	31.1 ± 8.5
Gender, No. (%)	
Male	147 (86.5)
Female	23 (13.5)
Legal status, No. (%)	
Undocumented	111 (65.3)
Refugee	59 (34.7)
Geographical origin, No. (%)	
Northern Africa	3 (1.8)
Sub-Saharan Africa	136 (80)
Eastern Europe	17 (10)
India-Pakistan subcontinent	13 (7.6)
South America	1 (0.6)
Residence in Italy, mo (mean ± SD)	32.4 ± 8.5
Years of schooling, mean ± SD	6.3 ± 4.2
Alcohol intake, No. (%)	25 (14.7)
Subjects declaring, No. (%)	
Drug addiction	0
Unsafe sexual activity	40 (23.5)
Surgery/dental care/abortion	73 (42.9)
Blood transfusion	2 (1.2)
Inapparent parenteral exposure[a]	168 (98.8)
None declared	2 (1.2)

[a]The term "inapparent parenteral exposure" includes unsafe therapy injection, acupuncture, tattoo, piercing, and tribal practices.

jects, the HBV genotype was detected in 109 and undetectable in 4, due to the low viral load. Of these 109, 75 (68.9%) had HBV genotype E, 18 (16.5%) genotype A, 13 (11.9%) genotype D, and only 3 (2.7%) genotype C, prevalently reflecting the HBV genotype distribution of their areas of origin.

The characteristics of the 170 HBsAg-positive subjects according to HBV genotypes are shown in Tables 2 and 3.

Seventy-five subjects with HBV genotype E who lived in Italy for a mean period of 43.2 ± 57.9 months and were prevalently from sub-Saharan Africa (93%), prevalently male (69%), and undocumented immigrants (64%). Genotype E was detected also in 3 immigrants from Eastern Europe, in 1 from the India-Pakistan subcontinent, and 1 from South America (Table 2).

All stated inapparent parenteral exposure, 25.3% unsafe sexual activity, 53.3% surgery/dental care/ abortion, and none stated drug addiction or blood transfusion (Table 2). Subjects with HBV genotype E showed an HBV-DNA serum concentration of 1.1E8 ± 8.9E8 IU/mL, 41% of them presented HBV-DNA > 2000 IU/mL, 21.3% were HBeAg-positive, and none circulated anti-HDV. Fifty-two (69.3%) subjects with HBV genotype E were HBV inactive chronic carriers, 17 (22.7%) had HBV-related chronic hepatitis, and 6 (8%) had HBV-related liver cirrhosis, 2 of whom with superimposed multifocal HCC (Table 3).

The 18 patients with genotype A were prevalently young male undocumented immigrants with a low level of schooling, who lived in Italy for 36.1 ± 38.4 months and were mostly from sub-Saharan Africa (61%) (Table 2). Also, in this subgroup the inapparent parenteral exposure was the most frequently stated risk factor. These subjects had a mean viremia of 5.8E7 ± 2.4E8 IU/mL, half of them had HBV DNA lower than 2000 IU/mL and were HBeAg-positive; 10 were considered HBV inactive chronic carriers, 7 had chronic hepatitis, and 1 had liver cirrhosis (Table 3).

The 13 immigrants with genotype D lived in Italy for 21.3 ± 22.8 months, were prevalently male, frequently with a low level of schooling, prevalently undocumented immigrants from Eastern Europe (38.5%) or the India-Pakistan subcontinent (30.8%). These subjects showed a low mean viremia (2.0E4 ± 3.9E4 IU/mL) and 53.8% had HBV DNA under 2000 IU/mL. All 13 were HBeAg-negative/anti-HBe-positive and none circulated anti-HDV. Eight subjects were HBV inactive chronic carriers, 5 had chronic hepatitis B, and none had liver cirrhosis.

Three subjects with HBV genotype C were young males from the India-Pakistan subcontinent; they were undocumented immigrants who lived in Italy for 8.7 ± 3.0 months. Of these 3, two subjects showed viremia over 2000 IU/mL and 1 circulated HBeAg; 1 subject was an HBV inactive chronic carrier, 1 had chronic hepatitis, and 1 had liver cirrhosis.

Of the 170 HBsAg-positive subjects, 1 was HBsAg/anti-HIV/anti-HCV-positive with undetectable HBV DNA, 4 were HBsAg/anti-HIV-positive (3 with HBV genotype E and 1 with very low viremia and undetectable HBV genotype), and 5 were HBsAg/anti-HCV-positive of whom 4 with undetectable HBV genotype and 1 with HBV genotype D. Thus, only 4 of these 10 HBsAg-positive patients with HIV and/or HCV coinfection had a detectable HBV genotype.

5. Discussion

HBV genotype E shows a low degree of genetic diversity in blood samples collected from Senegal to Angola, supporting the segregation of this genotype into monophyletic groups (30), its relatively recent introduction to

Table 2. Characteristics of the Studied Immigrants with HBsAg-Positive and Chronic Liver Disease, According to Hepatitis B Virus Genotype

HBV Genotype	A, A1	C	D, D1, or D2	E	E vs. A, P value	E vs. D, P value	A vs. D, P value
Number of patients	18 (16.5)	3 (2.7)	13 (11.9)	75 (68.9)			
Gender, No. (%)					0.65	0.12	0.62
Females	2 (11)	0	3 (23.1)	6 (8)			
Males	16 (88.9)	3 (100)	10 (76.9)	69 (92)			
Age, y (M±SD)	29.2 ± 5.9	29±48.5	34.8±11.1	30.8±7.7	0.41	0.11	0.08
Legal status, No. (%)					0.10	0.2	0.67
Undocumented	13 (72.2)	3 (100)	11 (84.6)	48 (64)			
Refugee	5 (27.8)	0	2 (15.4)	27 (36)			
Geographical origin, No. (%)					0.001 sub- Saharan vs. others	0.0001	0.08
Northern Africa	2 (11)	0	1 (7.7)	0			
Sub-Saharan Africa	11 (61.1)	0	3 (23.1)	70 (93.3)			
Eastern Europe	5 (27.8)	0	5 (38.5)	3 (4)			
India-Pakistan subcontinent	0	3 (100)	4 (30.8)	1 (1.3)			
South America	0	0	0	1 (1.3)			
Residence in Italy, mo (mean ± SD)	36.1±38.4	8.7±3.0	21.3± 22.8	43.2±57.9	0.63	0.19	0.23
With risk factors, No. (%)					0.01 unsafe sexual habits vs. others	0.1	0.06
drug addiction	0	0	0	0			
unsafe sexual activity	0	1 (33.3)	3 (23.1)	19 (25.3)			
surgery/dental care/abortion	11 (61.1)	1 (33.3)	9 (69.) 2	40 (53.3)			
Blood transfusion	0	0	1 (15.4)	0			
[a] **Inapparent parenteral exposure**	18 (100)	3 (100)	13 (100)	75 (100)			
None declared, No. (%)	0	0	0	0			

[a] Inapparent parenteral exposure includes unsafe therapy injection, acupuncture, tattoo, piercing, and tribal practices.

Central/Western Africa, and its explosive spread in these geographical areas through unsafe re-using of glass syringes and needles during mass-vaccination campaigns against yaws, sleeping sickness, smallpox, and measles (32, 33). The data of the current paper confirmed this spread; out of the 109 HBsAg-positive immigrants with a detectable HBV genotype, 84 were from sub-Saharan areas and of these, 70 (83.3%) carried HBV genotype E. Another 5 patients showed HBV genotype E in the current study, 3 from Eastern Europe, 1 from the India-Pakistan subcontinent, and 1 from South America. These observations, together with a report from Colombia, showing HBV genotype E in nine HBV-infected subjects in an Afro-American community (34), suggested that appropriate prophylactic measures should be taken to limit the spread of this viral genotype.

Nearly 70% of patients with HBV genotype E in the current study had an inactive CLD, most of whom with an HBV-DNA serum concentration lower than 2000 IU/mL, almost 23% had chronic hepatitis and 8% had liver cirrhosis. Compared to the patients with CLD and HBV genotype A or D, the ones with HBV genotype E more frequently had cirrhosis and HCC.

However, due to the low number of investigated patients with HBV genotype A or C, the possibility of a more aggressive role of HBV genotype E in disease progression needs confirmation in larger scale investigations. Coinfection with HIV or HCV occurred only in 3 of the 75 HBsAg-positive patients with HBV genotype E, and in 1 of the 13 with HBV genotype D; therefore, it only marginally influences the clinical presentation of these 2 subgroups of patients.

Considering all 170 HBsAg-positive subjects in the cur-

Table 3. Clinical and Virological Characteristics of the Studied Immigrants with HBsAg-Positive, According to Hepatitis B Virus Genotypes

HBV Genotype	A	C	D, D1, D2	E
Number of patients	18	3	13	75
HBV DNA IU/mL (M±SD)	5.8E7 ± 2.4E8	2.7E5 ± 4.7E5	2.0E4 ± 3.9E4	1.1E8 ± 8.9E8
HBV DNA < 2000 IU/mL	9 (50)	1 (33.3)	7 (53.8)	44 (58.7)
HBV DNA ≥ 2000 IU/mL	9 (50)	2 (66.6)	6 (46.1)	31 (41.3)
HBeAg-positive, No. (%)	9 (50)	1 (33.3)	0 (0)	16 (21.3)
Anti-delta-positive, No. (%)	0	0	0	0
AST, highest NV 40 IU/L, mean ± SD	32.1 ± 22.1	110.9 ±126.1	37.3 ± 22.7	52.7 ± 63.5
ALT, highest NV 40 IU/L, man ± SD	39.1 ± 25.7	153.0 ± 180.0	15.0 ± 45.0	57.6 ± 80.8
With inactive chronic liver disease, No. (%)	10 (55.5)	1 (33.3)	8 (61.5)	52 (69.3)
With chronic hepatitis, No. (%)	7 (38.9)	1 (33.3)	5 (38.5)	17 (22.7)
With cirrhosis, No. (%)	1 (5.5)[a]	1 (33.3)[b]	0	[c]6 (8.0)

[a] vs.
[b] P value = 0.02.
[c] 2 patients with HCC.

rent study, nearly 80% showed an inactive CLD, less than one-fifth had chronic hepatitis, and approximately 3%had liver cirrhosis, a clinical presentation very different from that of observed in 513 HBsAg-positive Italian patients with CLD, investigated in 2014 (35), with only 3% of the subjects with an inactive CLD, nearly two-thirds with chronic hepatitis and one-third with liver cirrhosis. Indeed, the clinical presentation of the immigrants in the current study resembled more than observed in the authors' previous study on 700 HBsAg-positive patients with CLD collected in Italy from 1976 to 1981, half of whom had an inactive CLD (36).

Due to the progressive application of the universal HBV vaccination introduced in Italy in 1991 for all infants and all 12-year-old children (the latter limited to the first 12 years of the campaign), all Italians aged 0 to 33 years received vaccines against HBV by 2014.

Consequently, chronic hepatitis B is eliminated in the younger age groups and there is an age-related increase in cases with chronic hepatitis, cirrhosis, and HCC (35).

Immigration from the developing countries has become massive in Italy in the last 5 years and, consequently, the HBV genotype distribution is expected to change, particularly for genotype E, since most immigrants come from Central/Western Africa. It modifies the epidemiology and clinical presentation of HBV acute and chronic infection in Italy unless the Italian healthcare authorities apply effective prophylactic and therapeutic measures. Appropriate screening should be implemented to identify HBsAg-positive immigrants for educational, diagnostic, and therapeutic purposes, and the nationwide HBV vaccination campaign should be extended to the HBsAg-negative immigrant population. It was successfully done in pregnant immigrants who delivered in 2012/2013 at 8 Italian hospitals, where all new-born babies and all HBsAg-negative family members of the HBsAg-positive mothers were vaccinated against HBV (13). It is noteworthy that none of the 1727 immigrants evaluated in the current study and living in Italy for approximately 3 years had been vaccinated. The data of the current study confirmed that HBV screening for immigrants from geographical areas of high or intermediate endemicity was an important preventive measure for public health (37, 38).

Footnotes

Authors' Contribution: Evangelista Sagnelli, Loredana Alessio, Caterina Sagnelli, and Nicola Coppola, Literature reviewing, drafting the article, critically reviewed the manuscript and approving the final version; Luciano Gualdieri, Mariantonietta Pisaturo, Giovanni Di Caprio, Lorenzo Onorato, Margherita Macera, and Gaetano Scotto, data collection; Carmine Minichini and Mario Starace, performing the laboratory analyses.

Conflict of Interests: All the authors of the manuscript declared no conflict of interest.

Ethical Approval: The study was approved by the ethics committee of the Azienda Ospedaliera Universitaria of the Second University of Naples (protocol number 214/2012).

Funding/Support: The current study was supported in part by a grant from Gilead Sciences S.r.l. 'L'infezione da

HBV nelle popolazioni speciali (donne in gravidanza, popolazioni immigrate, popolazioni in eta pediatrica): progetti di awareness ed accesso alla diagnosi' Fellowship Program 2011 and 2013; and by a grant from 2014 goSHAPE program.

References

1. Zehender G, Ebranati E, Gabanelli E, Sorrentino C, Lo Presti A, Tanzi E, et al. Enigmatic origin of hepatitis B virus: an ancient travelling companion or a recent encounter? *World J Gastroenterol.* 2014;**20**(24):7622–34. doi: 10.3748/wjg.v20.i24.7622. [PubMed: 24976700].
2. Paraskevis D, Magiorkinis G, Magiorkinis E, Ho SY, Belshaw R, Allain JP, et al. Dating the origin and dispersal of hepatitis B virus infection in humans and primates. *Hepatology.* 2013;**57**(3):908–16. doi: 10.1002/hep.26079. [PubMed: 22987324].
3. Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology.* 2004;**47**(6):289–309. doi: 10.1159/000080872. [PubMed: 15564741].
4. Coppola N, Masiello A, Tonziello G, Pisapia R, Pisaturo M, Sagnelli C, et al. Factors affecting the changes in molecular epidemiology of acute hepatitis B in a Southern Italian area. *J Viral Hepat.* 2010;**17**(7):493–500. doi: 10.1111/j.1365-2893.2009.01201.x. [PubMed: 19780943].
5. Coppola N, Tonziello G, Colombatto P, Pisaturo M, Messina V, Moriconi F, et al. Lamivudine-resistant HBV strain rtM204V/I in acute hepatitis B. *J Infect.* 2013;**67**(4):322–8. doi: 10.1016/j.jinf.2013.06.006. [PubMed: 23796869].
6. Coppola N, Sagnelli C, Pisaturo M, Minichini C, Messina V, Alessio L, et al. Clinical and virological characteristics associated with severe acute hepatitis B. *Clin Microbiol Infect.* 2014;**20**(12):O991–7. doi: 10.1111/1469-0691.12720. [PubMed: 24930916].
7. Sagnelli C, Ciccozzi M, Pisaturo M, Zehender G, Lo Presti A, Alessio L, et al. Molecular epidemiology of hepatitis B virus genotypes circulating in acute hepatitis B patients in the Campania region. *J Med Virol.* 2014;**86**(10):1683–93. doi: 10.1002/jmv.24005. [PubMed: 24980631].
8. Sagnelli C, Ciccozzi M, Pisaturo M, Lo Presti A, Cella E, Coppola N, et al. The impact of viral molecular diversity on the clinical presentation and outcome of acute hepatitis B in Italy. *New Microbiol.* 2015;**38**(2):137–47. [PubMed: 25915056].
9. Zehender G, De Maddalena C, Giambelli C, Milazzo L, Schiavini M, Bruno R, et al. Different evolutionary rates and epidemic growth of hepatitis B virus genotypes A and D. *Virology.* 2008;**380**(1):84–90. doi: 10.1016/j.virol.2008.07.009. [PubMed: 18715605].
10. Sagnelli E, Pisaturo M, Martini S, Filippini P, Sagnelli C, Coppola N. Clinical impact of occult hepatitis B virus infection in immunosuppressed patients. *World J Hepatol.* 2014;**6**(6):384–93. doi: 10.4254/wjh.v6.i6.384. [PubMed: 25018849].
11. Sagnelli E, Starnini G, Sagnelli C, Monarca R, Zumbo G, Pontali E, et al. Blood born viral infections, sexually transmitted diseases and latent tuberculosis in italian prisons: a preliminary report of a large multicenter study. *Eur Rev Med Pharmacol Sci.* 2012;**16**(15):2142–6. [PubMed: 23280032].
12. Sagnelli E, Stroffolini T, Mele A, Imparato M, Sagnelli C, Coppola N, et al. Impact of comorbidities on the severity of chronic hepatitis B at presentation. *World J Gastroenterol.* 2012;**18**(14):1616–21. doi: 10.3748/wjg.v18.i14.1616. [PubMed: 22529690].
13. Sagnelli E, Taliani G, Castelli F, Bartolozzi D, Cacopardo B, Armignacco O, et al. Chronic HBV infection in pregnant immigrants: a multicenter study of the Italian Society of Infectious and Tropical Diseases. *New Microbiol.* 2016;**39**(2):114–8. [PubMed: 27196549].
14. Amini-Bavil-Olyaee S, Sarrami-Forooshani R, Mahboudi F, Sabahi F, Adeli A, Noorinayer B, et al. Genotype characterization and phylogenetic analysis of hepatitis B virus isolates from Iranian patients. *J Med Virol.* 2005;**75**(2):227–34. doi: 10.1002/jmv.20261. [PubMed: 15602742].
15. Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, et al. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol.* 1988;**69 (Pt 10)**:2575–83. doi: 10.1099/0022-1317-69-10-2575. [PubMed: 3171552].
16. Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol.* 2002;**83**(Pt 8):2059–73. doi: 10.1099/0022-1317-83-8-2059. [PubMed: 12124470].
17. Croagh CM, Desmond PV, Bell SJ. Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. *World J Hepatol.* 2015;**7**(3):289–303. doi: 10.4254/wjh.v7.i3.289. [PubMed: 25848459].
18. Centers for Disease Control and Prevention (CDC) . World hepatitis day fact sheet Available from: http://www.cdc.gov/Features/WorldHepatitisDay.
19. Hou J, Liu Z, Gu F. Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci.* 2005;**2**(1):50–7. [PubMed: 15968340].
20. European Commission . Migration and home Affairs Available from: https://ec.europa.eu/home-affairs/.
21. Caritas and Migrantes . XXIV Rapporto Immigrazione 2014, 1-25 Rome: Caritas and Migrantes; 2015. [cited 22 Aug]. Available from: http://www.caritasitaliana.it/materiali//Pubblicazioni/libri_2015/Rapporto_Immigrazione/sintesi_rapportoimmigrazione2014_quadroregionale.pdf.
22. Coppola N, Alessio L, Pisaturo M, Macera M, Sagnelli C, Zampino R, et al. Hepatitis B virus infection in immigrant populations. *World J Hepatol.* 2015;**7**(30):2955–61. doi: 10.4254/wjh.v7.i30.2955. [PubMed: 26730274].
23. Zampino R, Boemio A, Sagnelli C, Alessio L, Adinolfi LE, Sagnelli E, et al. Hepatitis B virus burden in developing countries. *World J Gastroenterol.* 2015;**21**(42):11941–53. doi: 10.3748/wjg.v21.i42.11941. [PubMed: 26576083].
24. El-Hamad I, Pezzoli MC, Chiari E, Scarcella C, Vassallo F, Puoti M, et al. Point-of-care screening, prevalence, and risk factors for hepatitis B infection among 3,728 mainly undocumented migrants from non-EU countries in northern Italy. *J Travel Med.* 2015;**22**(2):78–86. doi: 10.1111/jtm.12176. [PubMed: 25424439].
25. Sagnelli E, Sagnelli C, Pisaturo M, Macera M, Coppola N. Epidemiology of acute and chronic hepatitis B and delta over the last 5 decades in Italy. *World J Gastroenterol.* 2014;**20**(24):7635–43. doi: 10.3748/wjg.v20.i24.7635. [PubMed: 24976701].
26. Coppola N, Alessio L, Gualdieri L, Pisaturo M, Sagnelli C, Caprio N, et al. Hepatitis B virus, hepatitis C virus and human immunodeficiency virus infection in undocumented migrants and refugees in southern Italy, January 2012 to June 2013. *Euro Surveill.* 2015;**20**(35):30009. doi: 10.2807/1560-7917.ES.2015.20.35.30009. [PubMed: 26530499].
27. Filippini P, Coppola N, Pisapia R, Scolastico C, Marrocco C, Zaccariello A, et al. Impact of occult hepatitis B virus infection in HIV patients naive for antiretroviral therapy. *AIDS.* 2006;**20**(9):1253–60. doi: 10.1097/01.aids.0000232232.41877.2a. [PubMed: 16816553].
28. Gaiani S, Gramantieri L, Venturoli N, Piscaglia F, Siringo S, D'Errico A, et al. What is the criterion for differentiating chronic hepatitis from compensated cirrhosis? A prospective study comparing ultrasonography and percutaneous liver biopsy. *J Hepatol.* 1997;**27**(6):979–85. doi: 10.1016/S0168-8278(97)80140-7. [PubMed: 9453422].
29. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol.* 2001;**35**(3):421–30. doi: 10.1016/S0168-8278(01)00130-1. [PubMed: 11592607].
30. Mulders MN, Venard V, Njayou M, Edorh AP, Bola Oyefolu AO, Kehinde MO, et al. Low genetic diversity despite hyperendemicity of

hepatitis B virus genotype E throughout West Africa. *J Infect Dis.* 2004;**190**(2):400–8. doi: 10.1086/421502. [PubMed: 15216479].
31. Coppola N, Onorato L, Iodice V, Starace M, Minichini C, Farella N, et al. Occult HBV infection in HCC and cirrhotic tissue of HBsAg-negative patients: a virological and clinical study. *Oncotarget.* 2016;**7**(38):62706–14. doi: 10.18632/oncotarget.10909. [PubMed: 27486882].
32. Forbi JC, Vaughan G, Purdy MA, Campo DS, Xia GL, Ganova-Raeva LM, et al. Epidemic history and evolutionary dynamics of hepatitis B virus infection in two remote communities in rural Nigeria. *PLoS One.* 2010;**5**(7):e11615. doi: 10.1371/journal.pone.0011615. [PubMed: 20657838].
33. Andernach IE, Nolte C, Pape JW, Muller CP. Slave trade and hepatitis B virus genotypes and subgenotypes in Haiti and Africa. *Emerg Infect Dis.* 2009;**15**(8):1222–8. doi: 10.3201/eid1508.081642. [PubMed: 19751583].
34. Alvarado Mora MV, Romano CM, Gomes-Gouvea MS, Gutierrez MF, Carrilho FJ, Pinho JR. Molecular epidemiology and genetic diversity of hepatitis B virus genotype E in an isolated Afro-Colombian community. *J Gen Virol.* 2010;**91**(Pt 2):501–8. doi: 10.1099/vir.0.015958-0. [PubMed: 19846674].
35. Sagnelli E, Stroffolini T, Sagnelli C, Smedile A, Morisco F, Furlan C, et al. Epidemiological and clinical scenario of chronic liver diseases in Italy: Data from a multicenter nationwide survey. *Dig Liver Dis.* 2016;**48**(9):1066–71. doi: 10.1016/j.dld.2016.05.014. [PubMed: 27291331].
36. Giusti G, Ruggiero G, Galanti B. Chronic active hepatitis in Italy: a multicentric study on clinical and laboratory data of 1154 cases. A report from the study group for CAH of the Italian Association for the Study of the Liver. *Hepatogastroenterology.* 1983;**30**(4):126–30. [PubMed: 6629302].
37. Sharma S, Carballo M, Feld JJ, Janssen HL. Immigration and viral hepatitis. *J Hepatol.* 2015;**63**(2):515–22. doi: 10.1016/j.jhep.2015.04.026. [PubMed: 25962882].
38. Coppola N, Alessio L, Gualdieri L, Pisaturo M, Sagnelli C, Minichini C, et al. Hepatitis B virus infection in undocumented immigrants and refugees in Southern Italy: demographic, virological, and clinical features. *Infect Dis Poverty.* 2017;**6**(1):33. doi: 10.1186/s40249-016-0228-4. [PubMed: 28179020].