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RESEARCH ARTICLE

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Influence of universal HBV vaccination on chronic HBV infection in Italy: Results of a cross-sectional multicenter study

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Background and Aim: The universal hepatitis B vaccination for infants and 12-year-old adolescents (the latter limited to the first 12 years of application) was launched in Italy in 1991. Twenty-three years later we evaluated the impact of the vaccination campaign on the burden of HBsAg-positive chronic liver diseases (CLD).
Material and Methods: A total of 513 HBsAg-positive chronic carriers referring to 16 Italian liver units were investigated and compared with HBsAg carriers enrolled in previous surveys.
Results: The proportion of inactive carriers decreased from 20.0% in 2001 to 3.3% in 2014, while that of cirrhotic patients increased from 22.6% to 33.2%. Regarding the age class 0-33 (fully covered by HBV vaccination in 2014), the rate of inactive carriers decreased from the 21.7% in 2001 to 5.9% in 2014, that of chronic hepatitis from

Abbreviations: CLD, chronic liver diseases; HCC, hepatocellular carcinoma; AFP, α-fetoprotein; PCR, polymerase chain reaction.

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17.5% to 5.2% and that of cirrhosis cases from 26.4% to 4.1%. Instead, in the over-60 age group the rate of inactive carriers increased from 22.8% to 41.2% and that of chronic hepatitis from 16.8% to 46%; the rate of patients with cirrhosis ranged from 5% to 8% in different studies.

Conclusion: Twenty-three years after the introduction universal HBV vaccination in Italy, the clinical presentation of CLD had shown a shift toward older ages and more severe diseases.

KEYWORDS

chronic hepatitis B, HBsAg chronic carriers, HBsAg-positive chronic hepatitis, HBsAg-positive chronic hepatitis clinical presentation, HBV vaccination

1 | INTRODUCTION

Universal vaccination against HBV infection was introduced in Italy in the summer of 1991 for all infants and all 12-year-old adolescents (the latter limited to the first 12 years of the campaign). As a result, by 2014 virtually all Italians aged 0–33 years had been vaccinated against HBV. In the pre-vaccination era, two Italian multicenter retrospective surveys evaluated the etiology and clinical presentation of chronic liver diseases (CLD). The first found that 60% of 1154 patients enrolled from 1976 to 1981 were HBsAg-positive[1]; the prevalence was 31.3% in a second survey performed on 5461 patients with CLD enrolled from 1980 to 1989.[2] In 2001, 10 years after the introduction of HBV universal vaccination, a prospective multicenter investigation on 9997 Italian patients with CLD showed that 23.2% were HBsAg-positive.[3] The above-mentioned studies indicate a substantial decrease in the impact of HBV etiology on CLD cases investigated in Italy from 1976 to 2001.

In 2014 a further survey on 2557 subjects with CLD was performed.[4] The present paper describes the demographic and clinical data of the 513 subjects who were HBsAg-positive, and analyzes the changes in the demographics and clinical presentation occurring in Italy in the last 4 decades. More importantly, we analyzed the impact of universal HBV vaccination on the burden of HBsAg-positive CLD in Italy more than 23 years after its introduction.

2 | PATIENTS AND METHODS

The original database comprised all consecutive patients with CLD seen in 16 Italian liver units during the year 2014 as inpatients or outpatients. For the purpose of the present analysis, we retrieved all HBsAg-positive carriers aged over 18. Most of the participating liver units have cooperated in previous clinical investigations, and applied the same clinical approach and laboratory methods. The study design and method was described in detail in a previous study.[4]

The diagnosis of chronic hepatitis was based on liver histology in nearly 80% of the cases and on the persistence of ALT elevation for 6 months or more in the remaining 20% in the absence of biochemical and ultrasound or transient evidence of liver cirrhosis.[5] Liver cirrhosis was diagnosed based on liver histology or, in patients who did not undergo liver biopsy, on the presence of the peculiar clinical, biochemical, and ultrasound or transient signs.[5] According to accepted criteria, hepatocellular carcinoma (HCC) was diagnosed based on histological and/or imaging findings and on serum α-fetoprotein (AFP) levels.[6] The presence of serum HBsAg identified the HBV etiology, whereas the detection also of the antibody to HDV identified HBV/HDV coinfection and the detection of the antibody to HCV identified HBC/HCV coinfection; HBsAg positivity and alcohol abuse defined HBV/alcohol abuse. Alcohol abuse was defined as an intake of more than four alcoholic drinks per day over 5 years or more, whereas social drinking was a daily intake of 1-4 alcoholic beverages.[7,8]

An echo-assisted percutaneous liver biopsy was performed when required by the physician in care for diagnostic purposes. In each liver unit a skilled pathologist unaware of the clinical and laboratory data evaluated liver histology. In particular, liver necroinflammation and fibrosis were assessed by the Ishak[9] or Metavir scoring system[10] and standardized criteria were used to convert the Ishak scores to Metavir scores.[11] Transient elastometry was performed by Fibroscan[12–14] and used as an alternative method to assess liver fibrosis.[12]

Serum HBsAg and antibody to HCV, HDV, and HIV were sought using commercial immunoenzymatic assays. Plasma HBV DNA was determined by real-time polymerase chain reaction (PCR)[15] by this method, the detection limit in plasma samples is estimated at around 40 IU/mL.

The collection of personal data was made in full compliance with the Italian law on personal data collection, and each patient gave his/her informed consent to participate in the study, for the collection and storage of biological material and for the use in clinical research of the data obtained. All procedures applied in the study were in accordance with current international guidelines, with the standards of human experimentation of the local Ethics Committees, and with the Helsinki Declaration of 1975, revised in 1983. At the time of first observation, each patient signed an informed consent for the collection of personal data, established in full agreement with the rules of the Ethics Committee of the

coordinating center (A.O.U.P. of Palermo, Italy). Patients who agreed to undergo liver biopsy signed an appropriate informed consent before this procedure was performed. All patients were included only once, even if seen several times during the observation period. For each patient, demographic, etiological, and clinical data were recorded using a pre-coded questionnaire (Air-Tel Telematica, Italy). Due to the prevalent epidemiological nature of the study, data on the treatment of HBsAg-positive CLD were not collected. No patient refused to participate in the study.

2.1 | Statistical analysis

The data were collected in a pre-established electronic CRF database (web-based data collection, e-CRF provided by Air-Tel®, Airon Telematica, Milan, ITALY). Categorical variables were summarized as absolute counts and proportions. Differences in proportion were evaluated by the chi square test or Fisher's exact test. A P-value < 0.05 was considered to be significant. All P values were two-tailed.

3 | RESULTS

The 513 patients with HBV chronic infection enrolled in the present study were prevalently males (sex ratio = 1.8) and outpatients (88.5%), and 72.1% of cases came from southern Italy or one of the two major islands (Sicily or Sardinia). Eighty-four (16.4%) were alcohol abusers, 42 (8.2%) social drinkers, and 387 (75.4%) abstainers. Only 17 cases (3.3%) were HBV inactive carriers, 326 (63.4%) had chronic hepatitis, 118 (23.0%) had compensated cirrhosis, 28 (5.5%) decompensated cirrhosis, and 24 (4.7%) liver cirrhosis with superimposed HCC (Table1). The hepatitis B e antigen (HBeAg) was detected in 32 (6.2%) patients, anti-HDV positivity in 61 (11.9%), and anti-HCV positivity in 47 (10.7%) out of the 440 tested (Table 2). HBV-DNA values were not analyzed, because of the possible influence of anti-HBV treatments, which were not registered.

The original database consisted of 2557 patients with CLD, of whom the 513 (20.2%) with HBV etiology are described in the present study. The database included also 1438 cases with HCV etiology, alone in 1286 (50.3%) and with concomitant alcohol abuse in 152 (5.9%). Alcohol abuse was the only etiologic agent of 163 cases (6.4%), NAFLD/NASH of 162 (6.3%), autoimmune hepatitis of 60 (2.3%), and primary biliary cholangitis of 28 (1.1%); hemochromatosis and Wilson's disease were rare diagnoses. In the remaining patients no etiologic agent was detected.

To identify changes occurring in the last 4 decades, we compared the data from the present study with those observed in HBsAg-positive patients investigated in 1976-81, 1980-89, 2001, and 2006-2007 (Table 3). The mean age of patients progressively increased from 27 ± 16 years in patients enrolled in the 70s to 57.8 ± 13.7 in those observed in 2014, but male preponderance remained relatively stable over time. The data on the percentages of HBeAg, anti-HDV, and anti-HCV-positive cases were not available for patients enrolled in 1976-1981 or in 1980-1989. In subsequent studies from 2001 to 2014, the percentages of anti-HDV-positive subjects remained stable at around 11%, while the prevalence of anti-HCV-positive cases decreased from 16.8% in 2001 to 6.5% in 2006-2007 and then increased to 10.7% in 2014. The percentage of HBeAg-positive cases was 13.6% in the 2001 and 11.0% in the 2006/2007 cases, but it had dropped to 6.2% by 2014. The proportion of HBV inactive carriers progressively decreased over time from 46.9% to 3.3% (P < 0.01), that of patients with chronic hepatitis increased from 32.1 to 63.4% (P < 0.01), and that of patients with liver cirrhosis (with or without HCC) remained stable at around 20% from the 1970s to 2006-2007, but it had significantly peaked at 33.2% by 2014 (P < 0.01) (Table 3).

TABLE 1 Characteristics of the 513 HBsAg chronic carriers

Variables	
Age, years, M ± SD (range)	57.8 ± 13.7 (16-88)
Gender	
Male/female	330/183 (Sex ratio 1.8)
Referral pattern, *N*° (%)	
In-patients	59 (11.5)
Out-patients	454 (88.5)
Alcohol intake, *N*° (%)	
Abstinent	387 (75.4)
≤ 4 drinks/day	42 (8.2)
>4 drinks/day	84 (16.4)
Area of origin (birth), *N*° (%)	
North	65 (12.7)
Center	40 (7.8)
South/islands	370 (72.1)
Abroad	38 (7.4)
Years of awareness of HBsAg positivity	NA
Antiviral therapy	NA
Diagnostic category, *N*° (%)	
Inactive carrier	17 (3.3)
Chronic hepatitis	326 (63.4)
Compensated cirrhosis	118 (23.0)
Hepatocellular carcinoma	24 (4.7)
Decompensated cirrhosis	28 (5.5)

NA, data not available.

TABLE 2 Virological characteristics of all 513 HBsAg-positive patients

Viral markers	*N*°positive/*N*° tested	%
HBeAg-positive	32/513	6.2
Anti-HDV	61/513	11.9
Anti-HCV-positive	47/440[a]	10.7

[a]In 73 subjects the test was not performed.

TABLE 3 Characteristics of HBsAg chronic carriers in Italy before and after the vaccination campaign launched in 1991

Years of observation	1976-81 Ref. n° 1	1980-89 Ref. n° 2	2001 Ref. n° 3	2006-07 Ref. n° 28	2014 Ref. n° 10
Number of patients	700	1710	1336	1386	513
Age, years, M ± SD	27 ± 16	30.8 ± 17.2	49.1 ± 15.9	49.7 ± 12.8	57.8 ± 13.7
Males, %	75	74	71.3	73.4	64.3
HBeAg-positive, N° (%)	NA	NA	154/1133 (13.6)	148/1348 (11.0)	32 (6.2)
Anti-HDV-positive, N° (%)	NA	NA	61/723 (9.7)	95/1179 (8.2)	61 (11.9)
Anti-HCV-positive, N° (%)	NA	NA	205/1223 (16.8)	83/1277 (6.5)	47/440 (10.7)
Diagnostic category, N° (%)					
Inactive carrier	328 (46.9)	655 (38.3)	267 (20.0)	184 (13.3)	17 (3.3)
Chronic hepatitis	225 (32.1)	669 (39.1)	768 (57.5)	871 (62.8)	326 (63.4)
Cirrhosis	153 (21.9)	386 (22.6)	248 (18.6)	291 (21.0)	146 (28.5)
Cirrhosis plus HCC	NA	NA	53 (4.0)	40 (2.9)	24 (4.7)

NA, data not available.

Table 4 shows the distribution of the HBsAg-positive patients observed in the 2001, 2006-2007, and 2014 studies by age class (0-33, 34-59, and >60) and diagnosis (Table 4). The percentage of inactive carriers aged 0-33 years decreased from 21.7% in 2001 to 5.9% in 2014 and a similar downtrend was observed in patients with chronic hepatitis (from 17.5 to 5.2%), whereas the rate of cases with cirrhosis ranged from 5% to 8% in the different studies and no cirrhotic patient had superimposed HCC. Conversely, these percentages increased from 2001 to 2014 in the over-60 age sub-group (Table 4). To this regard, it should be stated that the universal HBV vaccination covered Italian citizens aged 0-10 and 12-21 in 2001, 0-26 in 2006-2007 and 0–33 in 2014, and that in the earliest years of application a 65.1% vaccine coverage had been registered in adolescents in some southern districts.

4 | DISCUSSION

One of the major aims of the HBV vaccination campaign was the reduction in the burden of HBsAg-positive CLD, including HCC. Population surveys performed in Taiwan on children born before and after the introduction of universal HBV vaccination showed a drastic decrease in the HBV carrier rate and infection rate among subjects born since the program began.[16] Moreover, studies on the secular trend of liver disease documented a 68% decline in mortality from fulminant hepatitis in infants and a 75% decrease in the incidence of HCC in children 6-9 years old after the introduction of a national vaccination program.[17] Similarly, substantial reductions in newly acquired HBV infections, HBsAg carrier rate and HBV-related mortality have been reported in countries where universal vaccination has been implemented, such as Alaska[18] and Gambia.[19]

In Italy, the data from the national surveillance system for viral hepatitis (SEIEVA) indicate a marked decrease in the incidence of acute B hepatitis in the age-groups covered by vaccination (Fig. 1). This is in good agreement with the results of a sero-epidemiological survey performed in 2010 in a Southern Italian town,[20] where no subject below 30 years was anti-HBc-positive, thus confirming the effectiveness of the vaccination program. Indeed, vaccination coverage in Italy ranged from 95% to 98% in different years of application.

Comparing the age distribution of chronic HBsAg carriers from the three different surveys, we evaluated the impact of vaccination on the burden of HBsAg-positive CLD over a 23-year period. The data showed a progressive, significant downtrend in the prevalence of chronic HBsAg carriers in the age group covered by vaccination.

The increasing coverage of HBV vaccination has minimized the proportion of young subjects with HBV-related CLD, generating over time an increase in the mean age of chronic HBsAg carriers. In the pre-vaccination era the mean age of patients with HBV-related CLD was 27.0 years in those enrolled in 1976-1981 and 30.8 years in those investigated in 1980-1989. The mean age of these patients was 49.1 years in 2001, when HBV vaccination covered all Italian citizens aged 0-10 and 12-21, 49.7 in 2006-2007, when HBV vaccination covered the age group 0-26, and 58.9 years in 2014, when virtually all the Italian citizens aged 0-33 were covered by HBV vaccination. However, 5.9% of HBsAg inactive carriers, 5.2% of patients with chronic hepatitis, and 4.1% of those with cirrhosis who eluded vaccination is still present in the age group 0-33 years. The low vaccine coverage (65.1%) among adolescents in southern areas of the country during the earliest years of the campaign, documented in a national survey performed in 136 Italian health districts,[21] may account for this.

The remarkable shift toward older age groups has generated important virological and clinical changes. The rate of HBeAg positivity has progressively declined in Italy from 58.4% detected in 356 patients investigated in 1975-1985[22] to 20.1% found in a multicenter study performed in 1990,[23] to 13.6% reported in 2001,[24] 11% in 2006-2007,[25] and 6.2% in the present investigation in 2014. Before the vaccination was introduced, HBV/HDV chronic coinfection was 23.4% in 1987[26] and 14.5% in 1992.[27] Subsequently, the prevalences ranged from 8.3% in 1997[28] to 11.9% in 2014,[29] mostly as a consequence of the impact of vaccination. Of note, all the anti-HDV-positive cases were over 50 years of age and half of them had cirrhosis; this pattern strongly supports the concept that HDV circulation has drastically decreased among Italian

TABLE 4 Distribution of HBsAg-positive patients investigated in 2001, 2006-2007, and 2014, by diagnosis and age, % by row

	2001 Glaxo study (N° = 1336)			2006-2007 study (N° = 1386)			2014 Epacron Study (N° = 513)		
Age class	≤33	34-59	≥60	≤33	34-59	≥60	≤33	34-59	≥60
N° of patients	197	813	326	183	892	311	24	246	243
Inactive carriers	58 (21.7)	148 (55.4%)	61 (22.8%)	37 (20.4%)	113 (61.3%)	34 (18.3%)	1 (5.9%)	9 (52.9%)	7 (41.2%)
Chronic hepatitis	134 (17.5%)	505 (65.7%)	129 (16.8%)	138 (15.8%)	585 (67.2%)	148 (17.0%)	17 (5.2%)	159 (48.8%)	150 (46.0%)
Liver cirrhosis	5 (2.0%)	146 (58.8%)	98 (39.2%)	8 (2.7%)	177 (60.8%)	106 (36.5%)	6 (4.1%)	68 (46.6%)	72 (49.3%)
HCC	0	14 (26.4%)	39 (73.6%)	0	17 (41.5%)	23 (58.5%)	0	10 (41.7%)	14 (58.3%)

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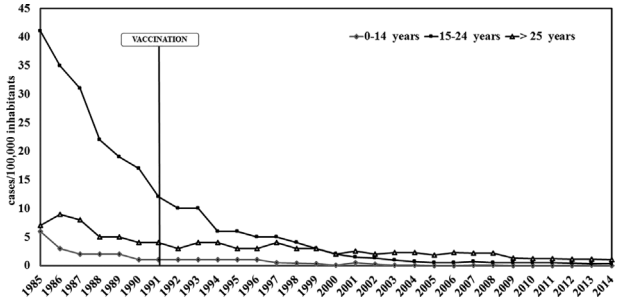

FIGURE 1 Age-specific incidence (n. cases × 100 000 inhabitants) of acute hepatitis B in Italy, 1985-2014. (Source of data: SEIEVA)

patients, and the current prevalence of anti-HDV positivity reflects a "survival effect"; HDV infection in Italy is an aging disease with a long-term course.

Surprisingly, the prevalence of HBV/HCV coinfection increased from 6.5% in 2006-2007 to the current 10.7%, a trend most probably due to a referral bias involving increased HCV testing at referral liver units due to the recent availability of free of charge second generation DAAs for HCV eradication.

In conclusion, this study provides evidence of the effectiveness of universal HBV vaccination in preventing chronic liver disease among the youngest generations 23 years after its launch in Italy.

CONFLICTS OF INTEREST

All the authors of this manuscript declare they have no conflicts of interest in connection with this paper.

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