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Vaccination against hepatitis B: Results of the analysis of 2000 population members in Croatia

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Abstract. The results of the analysis of vaccination against hepatitis B performed in 2,000 persons of high-risk groups in Croatia are described. All susceptible non-immunocompromised persons (HBsAg, anti-HBs, and anti-HBc negative) received either plasma-derived vaccine (HB-Vax MSD, 20 μg per dose) or recombinant HB vaccine (ENGERIX-B, SKB, 20 μg per dose) according to a 0,1 and 6-month schedule. Hemodialysis patients received four doses of HB vaccine (40 μg per dose). Seroconversion occurred in 98% of health care workers, 98.5% of family members of HBsAg chronic carriers, 98% of infants born to HBsAg carrier-mothers and 92% of hemodialysis patients. The percentage of poor-responders (titer of anti-BHs = 1–10 mIU/ml) for the groups was 2, 2, 8 and 20%, respectively, while low-responders (titer of anti-BHs = 10.1–100 mIU/ml) were 5, 4.5, 12 and 26%, respectively. A significant prevalence of non-responders, poor-responders and low-responders among male health care workers was noticed (p = 0.01, 0.026 and 0.002, respectively). Females significantly prevailed among excellent-responders (p = 0.0039). In hemodialysis patients, there were 8% non-responders, 19.5% poor-responders, and 26% low-responders. A significant difference between the percentage of good-responders (titer of anti-HBs = 101–1,000 mIU/ml) and excellent-responders (titer of anti-HBs over 1,000 mIU/ml) among health care workers and hemodialysis patients was documented (91% versus 46.5%, $p < 0.0001$). The combined passive-active immunization (hyperimmune hepatitis B globulin + hepatitis B vaccine) was effective in 98% of infants born to HBsAg carrier-mothers, and only one boy developed subclinical HBV infection (HBsAg and anti-HBc positive findings with normal ALT-values). Among these infants of seven months of age, a significantly higher percentage of poor-, and low-responders (20%) than that seen among older, non-immunocompromised persons (7% and 8% respectively) was noticed (p = 0.0241). Out of 20 initially non-responders, seroconversion occurred in 11 of them (54%) after two 'booster' doses of Engerix-B (20 μg per dose). By the use of the same procedure, a raised titer of anti-BHs was present in 92% of initially poor-responders and 100% of initially low-responders. Needle-stick exposure to HBsAg-positive blood occurred in 9 health care workers in the course of their vaccination and in 14 who had completed vaccination. Neither manifest nor subclinical HBV infections have developed among them. On the contrary, two surgeons who omitted the third dose of vaccine developed acute icteric hepatitis B two years later. Thus, complete vaccination (at 0, 1 and 6 months – 20 μg per dose) for non-immunocompromised persons gave good or excellent response in over 90% of recipients. In hemodialysis patients, the results are poorer irrespective of more and higher doses of HB vaccine.

Key words: Croatia, HBV infection, Prophylaxis

Introduction

Hepatitis B virus infection is one of the leading public health problems throughout the world [1–4]. The spectrum of clinical manifestations of HBV infection consists of acute hepatitis B (HB), asymptomatic chronic carrier state of HBV (HBsAg-positive persons), chronic persistent hepatitis B (CPH-B), chronic lobular hepatitis B (CLH-B), chronic active hepatitis B (CAH-B), cirrhosis of the liver (cirrhosis-B) and primary hepatocellular carcinoma of the liver (PHC-B) [1, 2, 5, 6]. Every year thousands of children and adults die due to HBV infection, especially fulminant HB, liver cirrhosis and PHC-B. There is not, to date, a completely effective therapy and treatment has been essentially symptomatic [2, 5, 7]. Active immunization using vaccine which contains HBsAg has been applied from 1981 (USA, France [2, 5]. According to a number of health organizations, anyone who has contact with human blood in their everyday life should be vaccinated [8–11]. This high-risk group consists of health care workers, children of chronic HBV carrier-mothers, family members of chronic HBV carriers, hemodial-

ysis persons, travelers in endemic areas of HBV infection, drug addicts, and homosexuals [2, 5, 6, 12–15]. The possibility of a past or ongoing HBV infection exists among these persons so pretesting is necessary before starting active immunization, except for travelers in endemic areas of HBV infection [11, 12, 14, 16, 17].

In the Republic of Croatia the use of active immunization started in 1987, but because there were no strict obligations and due to the expense, many candidates for vaccination have not yet been immunized. However, it is absolutely clear now that there has been decreased morbidity due to HBV among health care professionals in the district of Zagreb since vaccination started [18, 19]. By the correct use of HB vaccine from humanitarian aid, we believe that the frequency of HBV infection among high-risk groups throughout the Republic of Croatia will be reduced. The results of the analysis of 2,000 vaccinated persons from 1987 to 1991 are described in this article.

Materials and methods

Study population. The study population consisted of 2,000 members of high-risk groups for HBV infection in the Republic of Croatia (1,526 women and 474 men). Health care workers (1,359 women and 341 men) were recruited from general hospitals, family members of HBsAg chronic carriers (113 women and 87 men) were invited to participate, hemodialysis patients (25 women and 25 men) were recruited from three hemodialysis centers in Zagreb and infants of HBsAg carrier-mothers (29 girls and 21 boys) were identified through testing of the mother when pregnant. Family members of HBsAg chronic carriers included 60 adults (40 women and 20 men) and 140 children (73 girls and 67 boys) over two years of age.

HB immunization schedules. All the examinees were tested before immunization for HBsAg, anti-HBs, and anti-HBc (Ausria, Ausab, Corab assays, Abbott), except the infants born to HBsAg carrier-mothers. A total of 300 persons who were HBsAg-, anti-HBs, anti-HBc-positive were excluded from the final study population of 2,000 individuals at risk for HBV infection. All susceptible non-immunocompromised persons (HBsAg, anti-HBs and anti-HBc negative subjects) received three doses of HB vaccine according to a 0, 1 and 6-month schedule (20 μg per dose). A total of 450 received plasma-derived HB vaccine (H-B-Vax, MSD, one dose = 20 μg HBsAg) and 1,500 received recombinant HB vaccine (Engerix-B, SKB, one dose = 20 μg HBsAg). The family members of HBsAg chronic carriers and infants born to HBsAg carrier-mothers received only recombinant HB vaccine. All susceptible hemodialysis patients received four doses of HB vaccine at 0, 1, 2 and 6 month, with 40 μg HBsAg per dose. Among them, 24 received H-B-Vax, and 26 Engerix-B. The infants born to HBsAg carrier-mothers received the first dose of Engerix-B (20 μg per dose) in the gluteal region, and hepatitis B hyperimmune globulin (HBIG) on the opposite gluteal region immediately after birth. The second and third dose of vaccine were given one and six months later. Children over one year of age were vaccinated in the deltoid muscle.

One month after the third or fourth dose of HB vaccine the titre of anti-HBs was examined and expressed in mIU/ml (Ausab EIA, Abbott). Persons without seroconversion titer of anti-HBs were classified as non-responders, poor-responders had titers between 1–10 mIU/ml, low-responders from 10.1–100 mIU/ml, good responders between 101 and 1,000 mIU/ml and excellent-responders above 1,000 mIU/ml.

In 20 non-responders, 25 poor-responders and 25 low-responders two booster doses of Engerix-B (20 μg/ml per dose) were given. The first 'booster' dose was given six weeks after complete vaccination and the second one month later. One month after the application of the second 'booster' dose the titer of anti-HBs was re-examined.

Data analysis. The results of vaccination in all persons who completed vaccination according to schedule (0, 1 and 6 and 0, 1, 2 and 6 months), as well as age, sex, and type of HB vaccine were statistically analysed using the Chi-square test with Yate's correction and Fisher's exact test, two-tailed, for small numbers. The episodes of acute, icteric hepatitis B in two surgeons incompletely vaccinated are also described as well as accidental needle-stick exposures to HBsAg-positive blood during and after complete vaccination. The principle side-effects during the vaccination procedure are reported.

Results

As a result of the pretesting, a total of 300 (13%) individuals were excluded from the study. Of this, 1.8% of health care professionals were HBV chronic carriers (HBsAg and anti-HBc positive) and 11.2% had signs of previous HBV infection (anti-HBc and/or anti-HBs positive). Among chronic HBV carriers, males prevailed (62%). Only 9% of these persons had a history of jaundice. Among family members of chronic HBV carriers, 2.4% were HBV chronic carriers (79% males) and 23% had had previous HBV infection; no history of jaundice was reported. In hemodialysis patients, we detected 6% chronic HBV carriers (76% males) and 31% with previous HBV infection, with history of jaundice in 14% of them.

Table 1. The results of vaccination of health care workers

Titer of anti-HBs	H-B-Vax		Engerix-B		Total (%)		Total (%)
	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	
Non-responders (0)	2 (2)	4 (1)	11 (5)	17 (2)	13 (4)	21 (1.5)	34 (2)
Poor-responders (1–10 mIU/ml)	3 (3)	4 (1)	10 (4)	19 (2)	13 (4)	23 (2)	36 (2)
Low-responders (10.1–100 mIU/ml)	6 (5)	13 (4)	22 (10)	42 (4)	28 (8)	55 (4)	83 (5)
Good-responders (101–1,000 mIU/ml)	52 (46)	153 (45)	101 (44)	452 (44)	153 (45)	605 (44.5)	758 (45)
Excellent-responders (over 1,000 mIU/ml)	49 (44)	164 (49)	85 (37)	491 (48)	134 (39)	655 (48)	789 (46)
Total (%)	112 (100)	338 (100)	229 (100)	1,021 (100)	341 (100)	1,359 (100)	1,700 (100)

M = male; F = female.

The results of vaccination of 1,700 health care workers are presented in Table 1. Non-response in 2% of these non-immunocompromised persons and the significant prevalence of males were noticed ($p = 0.01$, Chi-square test). We also found a significant prevalence of men as poor- and low-responders ($p = 0.026$ and $p = 0.002$, Chi-square test, respectively). The geometric mean titer (GMT) for male poor-responders was 6 mIU/ml and 8 mIU/ml for females. For male and female low-responders, the GMT was 42 mIU/ml and 86 mIU/ml, respectively. Almost equally high percentages of good-responders (45%) and excellent-responders (46%) were found in this healthy population. Women were significantly greater in the group of excellent-responders ($p = 0.0039$, Chi-square test). The results achieved with H-B-Vax vaccine were better, but not significantly so, than with Engrix-B.

Table 2 shows the results of vaccination of 200 family members of HBsAg chronic carriers. The percent of non-responders (1.5%), poor-responders (2%), and low-responders (4.5%) who were male was greater, but not significantly so. The majority of these non-immunocompromised examinees were good 47.5%) or excellent-responders (44.5%). In the group of excellent-responders, females prevailed (51% versus 37%), but again not significantly.

The results of vaccination of 50 immunocompromised haemodialysis patients are shown in Table 3. Eight percent were non-responders, 19.5% were poor-responders, and 26% were low-responders, the differences in response between men and women were not significant. There were no excellent-responders in this group. The results with H-B-Vax vaccine were better than with Engerix-B, but not significantly so. A significant difference clearly exists between health care workers and hemodialysis patients in percentage of good and excellent response after complete vaccination (91% and 46.5%, respectively; $p < 0.0001$, Chi-square test).

In Table 4 the results of the vaccination of 50 infants born to HBsAg carrier-mothers are presented. We found one male infant did not respond (2%), being HBsAg- and anti-HBc-positive on final testing after complete vaccination, i.e., when he was seven months old. Thus, the combined passive-active protection of infants born to HBsAg carrier-mothers was effective in 98% of cases. There was a significantly higher percentage of poor- and low-responders among these infants (20%) than among the other non-immunocompromised groups (7% and 8%, respectively) ($p = 0.0241$, Fisher's exact test, two-tailed).

The influence of age and sex on the results of

Table 2. The results of vaccination of family members of BHsAg chronic carriers

Vaccine	Engerix-B		
Titer of anti-HBs	M (%)	F (%)	Total (%)
Non-responders (0)	2 (2)	1 (1)	3 (1.5)
Poor-responders (1–10 mIU/ml)	3 (3)	1 (1)	4 (2)
Low-responders (10.1–100 mIU/ml)	5 (6)	4 (3)	9 (4.5)
Good-responders (101–1,000 mIU/ml)	45 (52)	50 (44)	95 (47.5)
Excellent-responders (over 1,000 mIU/ml)	32 (37)	57 (51)	89 (44.5)
Total (%)	87 (100)	113 (100)	200 (100)

M = male; F = female.

Table 3. The results of vaccination of hemodialysis patients

Vaccine	H-B-Vax		Engerix-B		Total (%)		Total (%)
Titer of anti-HBs	M (%)	F (%)	M (%)	F (%)	M (%)F (%)		
Non-responders (0)	1 (8)	0	2 (15)	1 (8)	3 (12)	1 (4)	4 (8)
Poor-responders (1–10 mIU/ml)	3 (25)	1 (8)	4 (31)	2 (15)	7 (27.5)	3 (12)	10 (19.5)
Low-responders (10.1–100 mIU/ml)	3 (25)	3 (25)	3 (23)	4 (31)	6 (24)	7 (28)	13 (26)
Good-responders (101–1,000 mIU/ml)	5 (42)	8 (67)	4 (31)	6 (46)	9 (36)	14 (56)	23 (46)
Excellent-responders (over 1,000 mIU/ml)	0	0	0	0	0	0	0
Total (%)	12 (100)	12 (100)	13 (100)	13 (100)	25 (100)	25 (100)	50 (100)

M = male; F = female.

Table 4. The results of vaccination of infants born to HBsAg carrier-mothers

Vaccine	Engerix-B		
Titer of anti-HBs	Boy (%)	Girls (%)	Total (%)
Non-responders (0)	1 (4.8)	0	1 (2)
Poor-responders (1–10 mIU/ml)	3 (14.3)	1 (3.4)	4 (8)
Low-responders (10.1–100 mIU/ml)	3 (14.3)	3 (10.4)	6 (12)
Good-responders (101–1,000 mIU/ml)	12 (57.1)	22 (75.9)	34 (68)
Excellent-responders (over 1,000 mIU/ml)	2 (9.5)	3 (10.3)	5 (10)
Total (%)	21 (100)	29 (100)	50 (100)

vaccination of 1,950 non-immunocompromised persons are shown in Table 5. A significantly higher percentage of non-responders, poor-responders, and low-responders among males was evident, regardless of type of HB vaccine (18% versus 14%; $p = 0.004$, Chi-square test).

In Table 6 the influence of age on the results of vaccination with the two types of HB vaccine is presented. This significantly higher percentage of non-, poor- and low-responders in persons over 50 years of age was evident (46%, $p < 0.001$, chi-square test). There was a greater, but not significantly higher, percentage of good- and excellent-responders with H-B-Vax than with Engerix-B (85% and 82.5%, respectively) in all examinees; among persons over 50 years this difference was significant (54% versus 33%, $p < 0.001$, Chi-square test). The best results were obtained in groups of vaccinated persons between 30–39 years old (98 and 96%), and 20–29 years old (97 and 96%).

Table 5. The influence of age and sex on the results of vaccination*

Age group (years)	Number and percent with non-, poor and low response		Number and percent with good or excellent response		Total
	M (%)	F (%)	M (%)	F (%)	
0–4	7 (30)	6 (20)	16 (70)	24 (80)	53
5–9	4 (14)	3 (8)	25 (86)	33 (92)	65
10–19	1 (3)	1 (2)	30 (97)	40 (98)	72
20–29	2 (5)	8 (2)	36 (95)	312 (98)	358
30–39	7 (4)	16 (3)	164 (96)	505 (97)	693
40–49	16 (14)	32 (7)	97 (86)	420 (93)	565
Over 50	25 (58)	57 (56)	18 (42)	44 (44)	144
Total (%)	62 (18)	123 (14)	387 (82)	1378 (86)	1950

* Patients on hemodialysis excluded (n = 50).
M = male; F = female.

Table 6. The influence of age and the results of vaccination with plasma-derived and recombinant HB vaccine

	H-B-Vax	Engerix B		
Age group (years)	No. Total vaccinees*	Percent with good or excellent response	No. Total vaccinees	Percent with good or excellent response
0–4	–		53	75%
5–9	–		65	89%
10–19	–		72	97%
20–29	76	97%	282	96%
30–39	178	98%	515	96%
40–49	142	91%	423	89%
Over 50	54	54%	90	33%
Total (%)	450	(85%)	1500	(82.5%)

* Patients on hemodialysis excluded (n = 50).

The results of revaccination of non-, poor-, and low-responders with two 'booster' doses of Engerix-B are presented in Table 7. Out of 20 initially non-responsive persons, seroconversion occurred in 11 (54%). An increased titer of anti-HBs was noticed in 23 (92%) poor-responders and in 25 (100%) low-responders. The GMT in initially non-responders increased from 0 to 62 mIU/ml, in poor-responders from 6 mIU/ml to 122 mIU/ml and in low-responders from 62 mIU/ml to 174 mIU/ml.

Two susceptible surgeons received the first two doses of Engerix-B in April and May 1990. They neglected to receive the third dose of HB vaccine and they both developed acute, icteric hepatitis B, one (a 33-year old male) in July 1992 and the second (a 36-year old male) in September 1992. They completely recovered (normal ALT values) after two months, and HBsAg disappeared from their sera after three and four months. In both of these cases other causes of acute hepatitis were excluded (alcohol abuse, drug use, etc.). During five years of follow-up, 360 health care workers (15%) omitted their third dose of HB vaccine. To date none has developed acute, icteric hepatitis B, but unfortunately we do not know the rate of possible anicteric or asymptomatic HBV infection among them.

The main side-effects of the vaccination were localized pain at the site of inoculation (75%), nausea (12%), weakness (17%) and raised temperature to 37.8 °C (8%). All these symptoms were mild and lasted 12–24 hours. Severe side effects were not reported.

The greatest problems in our vaccination program were refusal of vaccination, 26% belonging to high-risk groups, and neglecting the intervals between doses, especially between the second and third doses of vaccine (21%). Those persons were excluded from this study.

In 9 health care workers, needle-stick incidents with HBsAg-positive blood occurred during the course of vaccination, but none of them developed either manifest (elevated ALT-values, HBsAg and anti-HBc-positive findings) or subclinical HBV infection (normal ALT-values, anti-HBc and/or HBsAg-positive findings) during a 10-month follow up.

Table 7. The results of revaccination of non-, poor- and low-responders with two 'booster' doses of Engerix-B

After complete vaccination Titer of anti-HBs	Non-responders (n = 20)	Poor-responders (n = 25)	Low-responders (n = 25)
Non-responders (0)	9 (46%)	None	None
Poor-responders (1–10 mIU/ml)	2 (10%) GMT 8	2 (8%)	None
Low-responders (10.1–100 mIU/ml)	8 (39%) GMT 68	13 (52%) GMT 82	6 (24%) GMT 88
Good-responders (101–1,000 mIU/ml)	1 (5%) GMT 120	10 (40%) GMT 170	19 (76%) GMT 198
Excellent-responders (over 1,000 mIU/ml)	None	None	

GMT = Geometric mean titer (mIU/ml).

In 14 health care workers with complete vaccination (good- and excellent-responders), needle-stick exposure to HBsAg-positive blood occurred. Neither manifest nor subclinical HBV infection developed among them in a 10-month follow up. In 7 of them (50%), titer of anti-HBs increased (GMT = 812 mIU/ml) in relation to their initial GMT titer (542 mIU/ml), while in the remining 7 health care workers (50%) no changes in anti-HBs titer were noticed.

Discussion

In general, the vaccination procedures against hepatitis B in high-risk groups of populations are very successful in non-immunocompromised persons because over 90% of vaccinees seroconvert [2, 4, 5, 9, 11, 13, 20]. The results are poorer in immunocompromised persons, irrespective of type of vaccine or schedule of vaccination [13, 15–17, 21–26]. The main problems in hepatitis B vaccination are still the high cost of the vaccination series as well as refusal by many persons from high-risk groups [8, 10, 18, 22].

Our results have confirmed the very low percentage of non-responsiveness to the 0.1 and 6 month schedule of vaccination (20 μg per dose) by intramuscular route in health care workers (1–5%), family members of HBsAg chronic carriers (1–2%), and in infants born to HBsAg carrier-mothers (0–4.8%). On the contrary, the percentage of non-responders was significantly higher in immunocompromised hemodialysis patients (8–15%), irrespective of more (0, 1, 2, 6) and higher (40 μg per dose) doses of HB vaccine.

Among health care workers we found a significant prevalence of males among non-, poor-, and low-responders (4%, 4% and 8%, respectively) in relation to females (1.5%, 2% and 4%, respectively). Women significantly prevailed among excellent-responders (48%) in comparison to men (39%). In the group of family members of HBsAg chronic carriers, the results are similar but without a significant difference between males and females. It should be emphasized that the total number of examinees in this group was almost ten-times less than in the group of health care workers.

One boy in the group of infants born to HBsAg carrier-mothers was not protected by the use of the combined passive-active immunoprophylaxis at birth. Although the number in this group of immunized infants is too small to allow definitive interpretation, the combined passive-active immunization would be the treatment of choice. Altogether the results were significantly poorer among these youngest members of our study population than in the older health care workers and family members, but clearly better than in the hemodialysis patients (78%, 91%, 92%, 46.5%, respectively).

As already mentioned, we noticed a high percentage of poor response to HB vaccine among hemodialysis patients (53.5%), and there were no excellent-responders. Different methods for improving vaccine performance through appropriate dosage and schedule as well as immunopotentiating procedures are needed for hemodialysis patients [1, 27]. In non-immunocompromised patients the influence of age on the results of vaccination was evident. The poorest results were obtained in infants, significantly more often in boys (33.4%) than girls (13.8%), as well as in persons above 50 years old irrespective of sex (57%). The best results were obtained among persons from 20–39 years of age (96–98%).

The results obtained with plasma-derived vaccine exceeded the results obtained with recombinant HB vaccine in haemodialysis patients and health care workers but this difference was not significant. In our non-responders seroconversion occurred in 54% after two 'booster' doses of Engerix-B, as well as an increase of anti-HBs titer in 92% of poor-responders and 100% of low-responders.

The appearance of acute, icteric hepatitis B in two incompletely vaccinated persons demonstrates the significance of the complete procedure. As we reported earlier, it seems that the use of HB vaccine alone after accidental exposure to HBsAg-positive blood is efficacious in protecting non-immunocompromised adults [7, 10, 18, 19]. During ten months of follow-up we did not detect either manifest or subclinical HBV infection among good- and excellent-responder health care workers after needle-stick exposure to HBsAg-positive blood. It is interesting to note that in only 50% of the needle-stick individuals did the anti-HBs titer rise after the incident.

Pretesting of high-risk persons for HBV infection by determining HBsAg, anti-HBs and anti-HBc before vaccination reveals chronic HBV carriers as well as those with previous HBV infection [8, 9, 11, 14]. We found 1.8%, 2.4% and 6% of chronic HBV carriers, with a significant prevalence of males, among health care workers, family members of HBsAg chronic carriers and hemodialysis patients, respectively; a history of jaundice was seen in 9%, 0% and 6% and previous HBV infection in 11.3%, 23% and 31%, respectively. These persons did not require vaccination but they needed the control of a hepatologist, as did their close family members [3, 6, 10, 14].

Unfortunately, we have the advice that, at least in adults, the immune status in relation to hepatitis B does not depend only on the level of circulating antibodies. The long term protection resides in the memory cells in the immune system. However, in the absence of any standardized current test of the memory cells or of the cellular component of immunity against hepatitis B, the checking of anti-HBs titer could be needed [28].

According to some authors, a vaccination strategy

that targets only high-risk groups of a population will not affect the general population's rate of HBV infections [1, 3]. If this is so, the problem of HBV infection will remain important in Croatia, particularly in areas with a great number of refugees from Bosnia where HBV infection has been endemic.

In conclusion, vaccination against hepatitis B is very effective in non-immunocompromised health care workers and family members of HBsAg chronic carriers; the poorer results are seen in infants born to HBsAg carrier-mothers, persons older than 50 years, and especially in hemodialysis patients. A 'booster' dose or doses of HB vaccine could be promising in initial non-responders, as well as among poor- and low-responders and it appears that needle-stick exposure to HBsAg-positive blood is not harmful for good- and excellent-responders.

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