IMMUNOGENICITY OF PLASMA DERIVED V ACCINE IN ETHIOPIAN HOSPITAL PERSONNEL

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ABSTRACT: To study the immunogenicity of plasma derived hepatitis B vaccine, serological markers (HBsAg, anti HBc and anti HBs) were determined in 432 hospital employees by the Hepanostika microenzyme linked immunoassay method (ELISA) using kits obtained from Organon Technika Laboratories (Holland). Three doses of Pasteur plasma derived vaccine (Hevac B), containing 5 mcg of HBsAg, were administered intramuscularly at one month intervals to 80 of the l02 marker negatives. A booster dose was given at one year. Hepatitis B markers (HBsAg, anti HBc and anti HBs) were determined at 4, 12, 13 and 24 months by the ELISA method. Titration for anti HBs were pertormed at T 4, T 12, and T 13 by the radio-immunoassay method. Of 80 vaccinees, 2 discontinued after the first injection. Sero-conversion to anti HBs occurred at 4 months in 57 of 80 (71.3%); at 12 months in 64 of 73 tested (87.7%) and at months 13 and 24 in 66 of 69 tested (95.6%). Protective levels of anti HBs were achieved in titrated sera collected from sero-converters in 89%, 88.5% and 100% at months,4, 12 and 13 respectively. No vaccinee developed any evidence of hepatitis B infection during the two years of follow up. A female developed generalized skin rash and a pregnant woman aborted, both following the first injection. We conclude that plasma derive<! hepatitis B vaccine administered to adult Ethiopian hospital personnel is highly immunogenic and protective with minimal side effects.

INTRODUCTION

Hepatitis B is a disease of worldwide dis tribution. Morbidity and mortality are due to both the acute disease and chronic sequelae, i.e. chronic hepatitis, cirrhosis and hepatocellular carcinoma. The extent of the infection varies with the geographic location; being the highest in Sub-Sahara Africa, China and South-East Asia (1,2).

Measures such as screening of blood for hepatitis B surface antigen (HBsAg) before transfusion, the use of hepatitis B immunoglobulin following cutaneous or mucosal exposure to blood containing hepatitis B virus and similar preventive steps had only a small contribution to the overall control of hepatitis B infection. This leaves vaccination strategy as the single most important method of preventing hepatitis B infection.

Most sero-surveys conducted earlier have documented HBsAg carrier rate of more than 10% and an overall infection rate of greater than 70% in the adult population of Ethiopia (3,4,-5,6) even though some studies have reported lower rates among certain population groups (7,8). This clearly places it among the countries
with the highest prevalence. With this background knowledge it is obvious that only nationwide vaccination of all newborns will have a significant impact on the control of hepatitis B infection in this country. However, it also appears beneficial, even in hyper endemic to vaccinate target groups with an increased risk of infection among the adult population. Health care workers have been shown to belong to this group (5).

Even though several studies have shown the immunogenicity, efficacy and safety of both the plasma derived and recombinant DNA hepatitis B vaccines (9-17) we feel it is important to

document at least its immunogenic potential in Ethiopians before embarking on a large scale vaccination programme. It is with this in mind that we initiated this study in order to determine primarily the immunogenicity and simultaneously the tolerance of plasma derived vaccine among our hospital personnel.

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SUBJECTS AND METHODS

Participants

In September 1987, all employees of the Armed Forces General Hospital were invited to take part in the study. However, due to lack of diagnostics kits only the first 432 were selected and screened for HBsAg, antibody to coreantigen (anti-HBc) and antibody to surfaceantigen (anti HBs) by the Hepanostika microenzyme linked immunoassay method (ELISA) using commercially available kits obtained from Organon Technika Laboratories. One hundred and two marker negatives were eligible but 20 were unable to participate for various reasons and only 82 were enroled in the study. There were 24 males and 56 females with a mean age of 30.) (range 17-50) years.

Conduct of the study

Three doses of Hevac B Pasteur were administered intramuscularly into the deltoid muscle, at one month intervals, starting from the time of initial screening for hepatitis B viral markers (months, To, Ti, and TJ. A booster dose was given at 12 months (f IJ from the first injection. Before vaccine administration, verbal consent was obtained after explaining to each participant the possible side effects of the vaccine and the right to withdraw at any time of the study.

The vaccine is prepared from the plasma of healthy carriers of HBsAg and contains a highly purified and inactivated suspension of the sub-types "ad" and "ay" absorbed on to aluminium hydroxide. It also contained the preS protein which is the outermost epitome on the hepatitis B viral envelope. The vaccine is in a ready to use syringe containing 5mcg of HBsAg/ml and was kept at 2°C to 8°C until administration.

Seven ml of venous blood was obtained from each participant at T 4' T 12' T 13 and T 24 and the serum sent to the National Research Institute of Health for the determination of HBsAg, antiHBc and anti HBs. Sera obtained at To was sent to "Pasteur Vaccines" for the determination of hepatitis B markers (HBsAg anti HBc, anti HBs) while that of T 4' T 12 and T 13 were for the titration of anti HBs and anti PreS. Radio-immunoassay (RIA) was used for anti HBs and the ELISA method for anti preS titer determination. The results of anti HBs titration were expressed in international milli-units per millilitre (mIU/ml). Titers above 2 rnlU/ml were considered sero-conversion where as levels more than 10 mIU/ml were taken as protective. Anti preS values of 30 rnlU/ml or more were regarded as significant.

All sera were frozen and stored at 20°C before testing. In order to verify laboratory reliability 63 masked replicates of sera from T4 were sent to "Pasteur Vaccines", Paris, for the analysis of hepatitis B markers.In order to monitor the tolerance of the vaccine each participant was instructed to contact the principal investigator for any side effect and were also questioned about side effects every time they came for blood drawing.

Statistical Methods: Chi-square and Z-tests are used for differences between proportions.

RESULTS

Out of the 82 participants enroled in the study 80 received three doses of vaccine and 73 took the booster dose. Two subjects withdrew from the study after the first injection due to presumed side effects.

Sero-conversion to anti HBs occurred at 4 months in 57 of 80 (71.3%), at l2 months in 64 of73 tested (87.7%) and at 13 months in 66 of 69 tested (95.6%). At 2 years the anti HBs positivity rate remained unchanged in the 69 participants tested. This is shown in figure 1. Seven participants at T 12, 11 at T13 and T 24 were not available for testing. Three women followed for 2 years failed to sero-convert. Analysis for

Table 1. Sero-conversion rate by Age

Age bracket	Sero-conversion rate different months from first injection					
	T_4		T_{12}		T_{13}	
	No	%	No	%	No	%
< 20	(3/4)	75	(3/3)	100	(3/3)	100
21-30	(34/46)	73.9	(38/40)	95	(39/40)	97.5
31-40	(17/24)	70.8	(19/24)	79.17	(19/20)	95
41-50	(3/6)	50	(4/6)	66.66	(5/6)	83.33
Total	(58/80)	71.3	(64/73)	87.67	(66/69)	95.65

anti preS has been left out because most of the sera sent to "Pasteur Vaccines" were said to be inadequate for antibody determination. There is no statistically significant difference in sero-conversion rate between the different age brackets and the sexes (X2 and Z tests respectively). This is seen in tables 1 and 2.

Table 2. Sero-conversion rate by sex.

Months after first injection	Male		Female		Total	
	No	%	No	%	No	%
T_4	17/24	70.8	40/56	71.4	57/80	71.3
T_{12}	19/21	90.5	45/52	86.5	64/73	87.7
T_{13}	20/20	100	46/49	93	66/69	95.5

In the sero-converters titrated for anti HBs, a protective level was reached jn89%, 88.5% and 100% at months 4, 12 and 13 respectively. Statistical analysis using the Z-test did not show significant difference between the sexes in attaining this level of antibody, as shown in table 3.

The distribution of antibody titers in sero-converters, at the different time intervals is depicted in table 2. It is worth noting that there is a large increase in the levels of antibody titers

Table 3. Anti HBs above protective level in sero-converters titrated at different time intervals and distribution by sex

Months after first injection	No. titrated			No. with protective level of anti HBs & % protected of titrated		
	M	F	Total	M	F	Total
T_4	11	34	45	10(91)	30(88)	40(89)
T_{12}	12	40	52	9(75)	37(93)	46(89)
T_{13}	8	37	45	8(100)	37(100)	45(100)

following the booster dose. Geometric mean titer (GMT) in sero-converters were 14.64 at 4 months, 23.24 at 12 months and 1036.5 mIU/ml at 13 months.

No vaccinee came up with hepatitis B viral markers suggestive of acute hepatitis during the 24 months of observation. During the two years of follow up no side effects were reported except in the two women who discontinued the vaccination programme after the first injection. One developed a generalized macular skin rash, headache and fever on the same day as the vaccination and the other women aborted at 6 weeks of pregnancy, seven days after the injection.

There was complete agreement between the results obtained from the same sera tested both locally and at "Pasteur Vaccines" indicating reliability of the two laboratories.

DISCUSSION

This study demonstrates that plasma derived Pasteur Vaccine is highly immunogenic in a sample of adult Ethiopian health care workers. The 95.6% sero-conversion rate obtained one month after the booster dose is similar to results obtained by other investigators among different population groups, following varK>us vaccination protocols (10,12,14,16). It is worth noting that out of the 432 hospital employees screened only
102 had no evidence of hepatitis B infection. This has an important bearing on future strategies of vaccination programme in this country. In line with Gebreselassie's study (18) the most reasonable approach in an hyperendemic area, like Ethiopia, would be to vaccinate all new-borns within the framework of the expanded programme of immunization. With the current reduced cost of the vaccine it seems appropriate
to vaccinate even the adult population without screening for viral makers whenever vaccination is deemed necessary .

The presence of pre-S proteins in the vaccine is thought to enhance the immunogenicity of the vaccine. These pre-S antigenic determinants being the outermost epitomes on the hepatitis B virus envelope are said to induce virus neutralizing antibodies before antibodies to the S-proteins develop and may provide early protection by blocking the attachment of HBV to liver cells (19). Even though these claims cannot be sub-stantiated from our study the good level of antibody obtained with the relatively low antigenic dose used in this vaccine may suggest the augmentative role of pre-S protein.

Currently it is believed that anti HBs levels above 10 mIU/ml are protective against hepatitis B infection (20). In our study this level is surpassed by 100% of the 45 seroconverters titrated at T 13. This indicated that the potential of the vaccine to protect against infection is high among our study population, similar to observations by others (10,12,14,16).

Even though previous studies have given inconsistent results (11,12,15) we found no statistically significant difference in sero-conversion rate between the sexes. However, there were 3 obese females who failed to develop anti HBs during the whole period of follow up. This is in agreement with observations made by other investigators (21). It is speculated that the presence of large amounts of fat tissue inhibits the interfacing of the vaccine and antigen recognizing lymphocytes (22). However, others have come up with evidence of a genetic prediliction for non response to hepatitis B vaccine (23). The relatively low vaccine dose used in our study may also contribute to the poor antibody response in these obese participants.

Our study also showed no significant difference in vaccine response between the different age brackets. However, there is a trend of inverse relation between antibody titer and age. The lack of a more definite relationship may be due to the small number of vaccinees above the age of 41.

Antibody persistence following vaccination is said to be directly related to the peak GMT of antibody. Even though there was an impressive increase in antibody titer one month after the booster dose, the peak GMT documented in this study is lower than reported by some investigators (11,12,22). The reason for this is not obvious but the low dose utilized in this trial may have a role. This has been shown to be the case by rug et al. in which they documented a lower GMT with the 5 mcg of HBsAg compared to the 10 and 20 mcg (22). The issue of antibody persistence is important in relation to the need and timing of a booster dose (24). Nevertheless, the Centre for Disease Control in Atlanta Georgia, currently recommends that no routine booster dose be given in adults and children with normal immune status within 7 years after vaccination (25).

Skin rash and fever as a side effect of hepatitis B vaccine has been documented by other as well (10,12). However, from the review of the available literature abortion has not been reported in vaccine recipients. Even though this could have been just a coincidence, caution should be exercised when pregnant women are considered for vaccination. Sore arm is the most frequent side effect reported in other studies (10,12,14).However, none of the participants in this study complained of it even after direct questioning. We conclude that plasma derived Pasteur Vaccine administered to adult Ethiopian hospital personnel is high immunogenic, with minimal adverse effects.

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