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A Booster Dose for Hepatitis B Vaccine should be Recommended by EPI in Saudi Arabia at Adolescence and Young Adulthood

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Abstract **Purpose**: In 1989, hepatitis B vaccine program was started in Kingdom of Saudi Arabia (KSA). Most of the workers tested immunological status of vaccinated peoples by booster doses but did not investigate their susceptibility to hepatitis B virus (HBV) infection. Our study aimed to evaluate the immune status against HBV in Saudis vaccinated with HBV vaccine and their rate of HBV infection. **Methods**: Random blood samples were collected from blood donors and quantitatively screened for the antibodies directed to HBsAg (anti-HBs). Data regarding percent coverage of hepatitis B vaccination and HBV infection in different age ranges in KSA from 2002-2013 were collected. **Results**: Anti-HBs was detectable in most of the age ranges under recommended protective level (10 mIU/ml). Vaccine coverage was 95.5-98%. The rate of infections with HBV increased with the increase in age and most infections occurred in persons aged >14 years age-old. **Conclusions**: The results achieved reflect the success of EPI in Saudi Arabia to cover all target age for hepatitis B vaccine. Most infections occurred in persons aged >14 years age-old. A booster dose at that age is required to prevent adolescent and young adult HBV acquirement.

Keywords Anti-HBs, Blood donors, Expanded program of immunization (EPI), HBsAg, Hepatitis B vaccine, Aseer, KSA

1. Introduction

Hepatitis B virus (HBV) is considered as an important cause of mortality and morbidity all over the world where it may lead to hepatic decompensation, cirrhosis and hepatocellular carcinoma (HCC). Yearly about 600,000 people die due to the consequences of HBV. Infection with HBV is preventable with the currently effective and safe vaccine which has been available since 1982 [1].

The course of hepatitis B may be variable to a far extent [2]. Infection with HBV has different clinical features depending on many factors such as the patient's age at infection, immunological status and the stage at which the disease is discovered. In acute form of hepatitis B, the incubation period after becoming infected is about 3-4 months, with a range of 6 weeks to 6 months. It usually occurs when the immune response is in a good situation. Symptoms and signs of disease usually last for several weeks and about 1-2% of persons with acute hepatitis B die from fulminant hepatitis [3, 4]. In chronic form of hepatitis B infected persons are with some sort of immunodeficiency and become a reservoir for the virus and a source of infection to others. Such persons often do not feel ill for decades after getting the infection. About 25% of persons chronically infected are during childhood and about 15% die of liver cancer or cirrhosis at older ages [3, 5]. The probability of developing symptoms of a new HBV infection is age-dependent. About 90% of perinatal HBV infections are asymptomatic. In contrary, newly infected young children at the age of 1-5 years old about 5-15% of them may show the typical manifestations of acute hepatitis but this percentage may increase to about 33–50 % in older children, adolescents, and adults. About 30-50% of children infected with HBV at an age between 1 and 4 years develop the chronic condition. Whilst about 30-50% of adults are symptomatic when first infected but only about 2-5% become chronically infected [3, 6].

The idea of vaccination against hepatitis B is considered as an effective mean to prevent cirrhosis and hepatocellular carcinoma allover the world [7-12].

The first hepatitis B vaccine was available in 1981 which produced by inactivating the harvested 22 nm HBsAg particles from plasma of chronic HBsAg carriers [13]. The recombinant hepatitis B vaccine consisting of purified HBsAg was first introduced in 1986 and has since replaced the plasma-derived hepatitis B vaccine. Recombinant

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hepatitis B vaccine was shown to have a very strong safety [14] and efficacy [15-19].

In 1991, there was a recommendation by the World Health Organization (WHO) that all countries with a high hepatitis B rate to implement a policy of universal HBV vaccination by 1997 [20]. Saudi Arabia has incorporated HBV vaccination into national immunization programs for infants since 1989.

For the prevention of perinatal and early horizontal transmission of HBV, a recommendation by the WHO for the universal administration of a birth dose of the recombinant hepatitis B vaccine within the first 24 hours of life was issued [21]. In the time that approximately 90% of infants being infected perinatally become chronic HBV carriers, chronic HBV infection risk decreases to 30% for children infected between ages one and four years and to less than 5% for persons infected as adults [3, 22]. To ensure maximum efficacy, the recombinant hepatitis B vaccine birth dose must be administered as soon as possible following birth, preferably within 24 hours [21].

The duration of protection after completion of a primary series during infancy is unknown but thought to extend into adulthood, and no additional doses are currently recommended [23]. But it was shown that half of those completed the primary series of vaccination lost the vaccine-induced antibodies within 5 years from vaccination [24].

Serum antibodies are a highly effective tool for protection against diseases. After being subjected to an antigen, the immune response generates memory B cells and antibodies secreting plasma cells. After many years of vaccination, antibodies level to a specific antigen reflects the number and activity of long-lived plasma cells which home to and survive forever the bone marrow producing antibodies with regular rate [25, 26]. Plasma cells do not respond to antigens but B cells can do. To generate the recall response of the same antigen, memory B cells to that antigen have high-affinity B cell receptors that after binding to that antigen for which they are specific, divides and differentiate into new plasma cells and produce high-affinity IgG antibodies to that antigen. Many workers [27, 28] have demonstrated that in HBV vaccinated individuals, both B and plasma cells are regulated independently and also functional memory B cells can be found in persons with plasma anti-HBsAg IgG below 10 mIU/ml [28-30]. This explains why previously vaccinated subjects without detectable serum antibodies are able to show anamnestic responses [31, 32].

Our study aimed to evaluate the immune status against HBV in Saudis who are expected that completed the primary series of HBV vaccine through expanded program of immunization (EPI) in KSA. In addition, to evaluate the rate of infection with HBV at different ages of theses immunized subjects.

2. Materials and Methods

2.1. Study Design and Donors Selection

Both donor selection and blood testing were done according to WHO recommendations [33] and Saudi Ministry of Health (SMOH). Blood was collected randomly from healthy blood donor volunteers, who voluntary were referred to Aseer Central Hospital and Abha General Hospital, Saudi Arabia, during the period from 2012 to 2013. General information were obtained from donors which included name, date of last donation and date of birth (age). Since 1990, SMOH does not issue certificate of birth unless the neonate is vaccinated, so we added to the questionnaire if the subjected vaccinated against HBV for those older than 23 years old. Pre-donation counseling which is essential to offer the donor an opportunity to self-defer if he/she thinks that he/she may be unsuitable for donation, answer the donor's questions and reassures him/her, obtain the donor's consent to donate his/her blood and explain the procedures to the donor. Criteria for exclusion included evidence of acute or chronic hepatitis, other chronic conditions, congenital or acquired immune disorder, transplantation of bone marrow, type of addiction and immunosuppressive therapy. Blood samples were collected in glass tubes, sera were separated and stored at -20 ℃ till use. Detection of antibodies against HBsAg in blood donors who are free from other blood transmitted pathogen was done to evaluate the immune status against HBV. Data regarding HBV infection of Saudis were also collected to determine the rate of HBV infection at different age ranges.

2.2. Serological Screening of Blood Samples

All blood specimens were serologically tested using Enzyme-Linked Immunosorbant Assay (ELISA) kit to detect hepatitis B surface antigen (HBsAg) (Murex HBsAg Version 3, DiaSorin, UK), antibodies directed to hepatitis B core antigen (HBcAb) (Murex HBc-Ab (total), DiaSorin), anti-hepatitis C virus antibodies (HCV-Abs) (Murex anti-HCV, version 4.0, DiaSorin), antibodies to human immunodeficiency virus 1 and 2 (HIV-1/2-Ab) (Murex HIV Ag/Ab Combination kit, DiaSorin), antibodies to human T-cell lymphotropic virus I and II (HTLV-I/II-Ab) (DiaSorin), anti-*Treponema pallidum* (syphilis) antibodies were done using ICE* Syphilis kit (DiaSorin), malaria (Optimal IT test, BIO-RAD).

2.3. Molecular Screening of Blood Samples

All samples were tested for the presence of HBV, HCV, and HIV nucleic acids by NAT using Roche COBAS®TaqScreen MPX (found in Aseer General Hospital). The test is a qualitative *in vitro* test for the direct detection of human immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, human immunodeficiency Virus Type 2 (HIV-2) RNA, HCV-RNA and HBV-DNA in

human plasma. This test is intended for use to screen samples of donations of human whole blood and blood components including source plasma. This test was done for all samples obtained from donors for donation.

2.4. Detection of Antibodies against HBsAg

Antibodies to hepatitis B surface antigen (anti-HBs) were screened in blood units free of viral, bacterial and protozoan infections. Detection of anti-HBs was done using enzyme immunoassay for qualitative/quantitative determination of antibodies to hepatitis B surface antigen in human serum and plasma (DIA.PRO, Italy).

2.5. Data Collection

Agents causing acute or chronic viral hepatitis including hepatitis B viral infection have been recorded in Saudi Arabia since 1990 [34] through SMOH. Data regarding percent coverage of hepatitis B vaccination and HBV infection in different age ranges in Saudi Arabia were collected, interpreted and analyzed. Here we collected these data from Health Statistical Year Books, SMOH, through 2002-2013 [35].

2.6. Statistical Analysis

The obtained data were expressed as mean±SD and statistical and correlation analyses were done using the one-way ANOVA followed by a posthoc LSD (Least Significant Difference) test. A P value < 0.05 was statistically significant. These statistical analyses were done using the Statistical Package for the Social Sciences for Windows (SPSS, version 17.0, Chicago, IL, USA).

3. Results

3.1. Screening of Blood Donors

Voluntary non-remunerated 7,267 blood donors (7,241 males (99.64%, median age of 30) and 26 females (0.36%, median age of 28)) were selected to donate their blood. The largest proportion (50.52%, $P \leq 0.001$) of donors was in ages between 21 and 30 years old with a median age of 26 years (Table 1). The nationality distribution of the donors was from 15 countries. Almost all donors were Saudis (95.13%).

Molecular and serological screening of blood samples resulted in positivity to different markers (Table 1 and 2).

Table 1. Serological marker after screening of accepted donors for donation at Aseer

Marker	Syphilis	HBsAg	HBcAb	HCV	HIV	HTLV	NAT
Syphilis	2	0	2	0	0	0	0
HIV	0	1	1	0	2	0	0
HCV	0	0	1	5	0	0	2
HBsAg	0	71	70	0	1	0	66
HBcAb	2	70	470	0	1	0	79
HTLV	0	0	0	0	0	0	0
NAT	0	66	78	2	0	0	88

Table 2. Distribution of serological markers and NAT among different age groups at Aseer

Age	Number	HBsAg	HBcAb		HCV	HIV	HTLV	RPR	NAT
(Years)			Saudi	Non-Saudi					
18	77	0	0	0	0	0	0	0	0
19	124	0	2	0	0	0	0	0	0
20	228	0	5	0	0	0	0	0	0
21	241	0	3	0	0	0	0	0	0
22	300	2	7	1	1	1	0	0	1
23	370	3	4	1	0	0	0	0	3
24	377	0	8	3	0	0	0	0	15
25-30	2388	14	97	9	0	0	0	0	32
31-40	2203	23	124	43	1	1	0	2	32
41-50	786	24	102	19	0	0	0	0	5
51-60	173	5	39	3	0	0	0	0	0
Total	7267	71	391	79	2	2	0	2	88

Table 3. Levels of anti-HBs in different age groups at Aseer

Age (Years)	Total number	N0n-Saudis	Saudis		% of Saudis >10 IU
			HbsAb <10 IU	HbsAb >10 IU	
18	77	2	60	15	19.48
19	124	1	89	34	27.42
20	228	5	188	35	15.35
21	248	4	217	27	10.89
22	293	6	167	120	40.96
23	370	8	233	129	34.85
24	377	18	209	150	39.79
25-30	2388	129	1361	898	37.61
31-40	2203	127	1673	403	18.29
41-50	786	54	N/A	N/A	N/A
51-60	173	0	N/A	N/A	N/A
Total	7267	354	4197	1811	

N/A: not assayed

Two (0.028%, 33 and 36 years old) positive cases to anti-syphilis antibodies accompanied with positivity for HBcAb. Two (0.028%) positive cases to HIV-1/2, one is 22 years old with HBsAg and HBcAb positivity and the other is 33 years old and both were NAT negative. HCV-Ab were found in 5 (0.069%) volunteers, 1 (0.014%) case positivity to HbcAb and 2 (0.028%, 36 and 45 years) were positive for HCV-RNA as confirmed by PCR. There were 71 HBsAg positive volunteers, 70 of them were associated with HbcAb and one for HIV. Cases positive for HBsAg and HBV-DNA by NAT were 66. Cases positive for HBcAb and HBV-PCR were 65 and negative for HBV-DNA by NAT were 5. There was no coinfection of HBsAg with HCV, syphilis or HTLV. Screening of samples for HbcAb resulted in 470 positive cases. Of these cases, there were 70 positive to HBsAg and one positive for HIV. Cases positive for HBcAb and HBV-DNA were 79 and negative for HBV-DNA by NAT were 391. There were no HBcAb cases companied with HTLV markers. There were no positive samples for HTLV-1/2 antibodies.

An 88 cases resulted from screening for HIV, HBV and HCV nucleic acid by NAT. Cases positive for NAT and other positive markers were as follow; 2 cases were positive to HCV-Ab, 66 cases were positive for HBsAg and 79 cases positive to HbcAb. There were 9 cases positive for NAT with on other markers.

Anti-HBs were found in all groups tested (Table 3), but the number of subjects having the value of protective level (>10 mIU/ml) is less than those of having antibody titer under the protective level (<10 mIU/ml).

3.2. Data Collection

Data available from SMOH published in Statistical Health Book is from 2002 to 2013. Throughout this period, 55,837 hepatitis B virus infections were recorded in KSA. The frequency of cases and prevalence per 100,000 population in KSA and Aseer region are shown in table (4). The rate of incidence declined from 26.32 at 2002 to 14.2 at the year 2013. No available data were obtained for years 2002-2005 and 2008 for Aseer region.

The incidence rate of HBV among male and female Saudis and non-Saudis is shown in table (5). The numbers of infected males in both Saudis and non-Saudis over number that of infected females. The reported HBV cases percent of non-Saudis decreased from 17.5% at year 2006 to 12% in the year 2013 and the incidence rate/100,000 cases decreased from 12.49 at the year 2006 to 5.4 at the year 2013.

Table 4. Reported cases and incidence rates of notifiable HBV in KSA and Aseer

Year	Number of reported cases (total in KSA)	Incidence rate /100,000 population	Reported cases in Aseer
2002	5638	26.31	N/A
2003	4329	19.65	N/A
2004	4594	20.34	N/A
2005	4209	18.20	N/A
2006	4264	18.20	201
2007	4501	18.57	232
2008	5066	20.43	296
2009	5020	19.78	329
2010	4854	18.72	393
2011	4494	15.84	411
2012	4609	15.84	457
2013	4259	14.2	281

Table 5. Reported Cases of HBV infection by Nationality and Sex through years 2006-2013 in KSA

Year	Saudis				Non-Saudis				Total	Rate (total)
	Male	Female	Total	Rate	Male	Female	Total	Rate		
2006	1886	1632	3518	19.89	527	219	746	12.49	4264	18.02
2007	1990	1684	3674	20.77	600	227	827	12.62	4501	18.57
2008	2376	1850	4226	23.35	613	227	840	12.50	5066	20.43
2009	2464	1897	4361	23.52	441	218	659	9.60	5020	19.78
2010	2305	1810	4115	0.0217	511	228	739	10.6	4854	18.72
2011	2129	1629	3758	19.37	485	251	736	8.2	4494	15.84
2012	2305	1613	3918	19.65	461	230	691	7.59	4609	15.97
2013	2300	1406	3706	18.41	383	140	523	5.40	4259	14.20

Rate: Incidence rate/100000 population

There was always HBV infection in all age groups with an increase in numbers as the age group increases except for age group 45+ the number of HBV-infected persons decreased (Table 6).

Table 6. Reported Cases of HBV infection by age group in KSA through years 2006-2013

Year	Age group					Total
	<1	1-4	5-14	15-44	45+	
2006	15	14	94	2891	1250	4264
2007	11	17	197	2947	1329	4501
2008	21	32	90	3482	1441	5066
2009	11	12	72	3528	1397	5020
2010	11	18	91	3402	1332	4854
2011	13	17	46	3115	1303	4494
2012	14	21	40	3104	1430	4609
3013	30	9	36	.2923	1234	4259

4. Discussion

Starting the vaccination against HBV at birth is proved to be a safe and effective means of preventing the infection with this virus at perinatal and childhood [36].

It was demonstrated that before 1990 the prevalence of HBsAg seropositivity in Saudi Arabian children up to 12 years old was on average of 6.7% and among adult reached 7.4% [37, 38]. Starting from 1990, the Saudi Arabian national strategy for the elimination HBV infection in the kingdom applied the universal administration of HBV vaccine to all infants. To ensure as much as possible coverage of vaccination against HBV the Saudi Arabian government conditioned the issuance of birth certificates upon completion of the first year vaccination program. The first dose of the HBV vaccine is administered at birth, the second at 1 month of age and the third at 6 months of age. In addition, children at school entry were also routinely administered a three-dose series of HBV vaccine from 1990 to 1995. Using this strategy, it is supposed that any individual who born in Saudi Arabia after 1985 are generally vaccinated against HBV.

In our study, prevalence of HBsAg infection in blood donors from Aseer was only among Saudis. This may be due to restricted criteria for non-Saudis to be employed in KSA which include health check for all transfusion-transmissible agents including HBV. The presence of HBcAb in some age groups of those non-Saudis indicates previous HBV infection. The incidence rate of HBV infection in Aseer during this study period was 0.98%. The previous study done by Madani [34] showed that prevalence of HBV infection in KSA was on an average of 0.15% at the period 1990-1999, while it was shown by Health Statistical Book published by SMOH that HBV infection is 0.18% during the period 2002-2013. Also in the same study, it was reported that Aseer is one of the high rate infection areas.

In our study, there was no HBsAg marker detected in ages 18-21 and 24 or any other markers except HBcAb. The presence of HBcAb may be due to as reported previously in vaccinated children at school level indication of natural infection and also a small number of children who had adequate immunization developed HBV infection [39].

If we compare the percentage of infected persons per groups in our study we can notice a repeated picture in studied years where there is an increasing order in HBV infection from group <1 year up to group 15-44 years and then a decrease in group 45+ years. If we compare the percentage of infected age groups with those of blood donors we can notice that these percentages are almost similar meaning that the reasons and ways of acquiring HBV infection may be the same all over the kingdom. There was a significant decrease (< 0.05) in percent infection in ages 5-14 from the year 2006 to 2013 which was not applicable to other age groups.

In a study done by Al-Faleh et al. [40] on vaccinated Saudi Arabian children up to 12 years old showed that the protective titer of anti-HBs (10 mIU/ml) was found in 77% of them and 71% in children at school entry. If we compare these results with those obtained in our study we can notice that the maximum protective titer was 40% (at age 22 years old) and minimum was 22% (at age 20 years old). This comparison indicates that the protective titer is going down with advances of age. So a booster dose at age 18 years old or older is required to prevent future acquirement of the HBV infection. In booster dose, it is better to change vaccine type to overcome the problem of non-responder subjects [41]. It was previously shown that a decrease in levels of anti-HBs occurs in most persons vaccinated at birth to levels less than the accepted threshold of protection (10 mIU/ml) 10 to 15 years after the primary vaccination series [42].

The primary vaccination with the recommended three doses of hepatitis B vaccine is expected to results in seroprotection in >95% of vaccinated infants and children. But after the completion of the primary vaccination series, anti-HBs concentrations decline and may fall below 10 mIU/ml after several years [29, 43-48]. In contrary, data from long-term follow-up studies in populations at ongoing risk for HBV infection showed that immunologic memory is able to prevent chronic or symptomatic infections even after anti-HBs declines to less than protective concentrations. Beginning vaccination at birth prevents perinatal and early childhood acquisition of HBV infection, and is expected to provide protection throughout adolescence and young adulthood [36]. Others studies on long-term immunogenicity of hepatitis B vaccine among persons aged 14–15 years who responded to a three-dose recombinant hepatitis B vaccine series starting at birth showed that anti-HBs concentrations decline over time and the majority of children of HBsAg-negative mothers do not have detectable anti-HBs 5–15 years after newborn immunization [23, 43-45, 47]. Hammitt et al. [23] showed in their study done on native Alaskan children that half of study participants had lost the ability to generate an anamnestic response following a booster dose of vaccine, suggesting possible loss of immunologic memory among some adolescents. In another

study, after vaccination with a primary series of 10 µg recombinant vaccine according to a usual (0, 1 and 6 months) or accelerated schedule (0, 1and 3 months) all infants achieved an anti-HBs response ≥10 mIU/ml, but this titer had fallen to <10 mIU/ml in one-third of those children at 5-years of age. All children who were given a booster vaccination responded and gave a titer of anti-HBs >100 mIU/ml [49].

The change in vaccine type may result in better response and memory. In study done by Bruce et al. [50] showed that Alaskan vaccinated with plasma-derived hepatitis B vaccine in 1981, and their antibodies went done protective level responded well to the new generation of vaccines. Zaffina et al. [41] concluded that in non-responder persons who received multiple immunizations with the same type of vaccine are inefficient in terms of antibody production and that re-vaccination may even be detrimental because it leads to a reduction of the frequency of specific memory B cells. So to boost persons the vaccine manufacturer or type must be changed to get better antibodies and memory cells response.

As previously indicated that people born in 1985 and later may get vaccinated in KSA [34], the presence of anti-HBs in people in our study aged more than 28 without the presence of neither HBsAg and anti-HBc indicates that either those peoples were vaccinated in adulthood as a result of public health awareness increase or cured from HBV infection without detectable amount of anti-HBc.

5. Conclusions

Expanded program of immunization in KSA against HBV could vaccinate and protect majority of infants and succeeded to protect most of the Saudi targets. HBV infection is going to be acquired by Saudis as age increase after vaccination. Booster dose of hepatitis B vaccine is recommended at adolescence and young adulthood and may be with different type of vaccine manufacturer.

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