Assessment of the Immune Status among Hepatitis B Virus Vaccinated Children in Jeddah City

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Abstract: HBV, a DNA virus transmitted percutaneously, sexually, and perinatally, affects 350 to 400 million persons worldwide. HBV was once considered hyper-endemic in the Kingdom of Saudi Arabia (KSA), where infection was acquired mainly through horizontal transmission early in life, and less commonly by vertical transmission similar to what is observed in other HBV-endemic countries. One thousand two hundred and seventy four (74.3%) Saudiand 382 (25.7 %) non-Saudi children were enrolled in this study along a period of 28 months at King Fahad General Hospital in Serology Lab. The objectives of this study was to evaluate Antibody response to Hepatitis B virus,which was investigated toevaluate the immune response to HBV vaccine among vaccinated children with the three doses of HBV vaccine by measuring the level of circulating anti-HB surface antibodies,and also to evaluate the long-term efficacy of hepatitis B virus immunization program among HBV vaccinated children in preventing hepatitis B virus "HBV" infection by measuring HBsAg, HBcIgG. Besides the finding of mutant HBV strains in the community, which can cause resistance to HBV vaccination. 1274/1274(100 %) of these samples were found seronegative for HBsAg,44/1274 (3.5 %) of these samples were found seropositive for HBcIgG, while 1224/1274 (96.1 %) of these samples were found seronegative for HBcIgG. 340/828 (41.1 %) of these samples were found seropositive for HBsAb, while 488/828 (58.9%) were found seronegative for HBsAb. Overall vaccinated responders, who were showing seropositivity with one marker only HBsAb,were 324/828 (39.1%). Meanwhile, 482/828 (58.21%) were showing seronegativity with all three markers. Based upon the present study, a serious alarm should paid and attention by the health authorities.Hepatitis B vaccine efficacy was not very high, and HBs Ag was not detected in any participant in the study, Based on the findings of our study there is a trend of decreasing antibody level, we recommend monitoring test of anti-HBs antibody for children to identify those who need a booster dose.

[Sanaa, G. Al Attas. **Assessment of the Immune Status among Hepatitis B Virus Vaccinated Children in Jeddah City**. *Life Sci J* 2013;10(3):2697-2706]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 389

Key words: HBV vaccine, children Serostatus HBV Vaccine,HBV vaccinated 1-18 children, HBcIgG, HBsAg, HBsAb, responders to HBV vaccine, non-responders to HBV vaccine.

1.Introduction:

HBV, a DNA virus transmitted percutaneously, sexually, and perinatally, affects 350 to 400 million persons worldwide. HBV infection accounts annually for 1 million deaths worldwide from cirrhosis, liver failure, and hepatocellular carcinoma (40,53,63,71,72)

Viral proteins of clinical importance include the envelope protein, hepatitis B surface antigen (HBsAg); a structural nucleocapsid protein, hepatitis B core antigen (HBcAg); and a soluble nucleocapsid protein, hepatitis B e antigen (HBeAg). Serum HBsAg is a marker of HBV infection, and antibodies against HBsAg signify recovery.

HBV was once considered hyper-endemic in the Kingdom of Saudi Arabia (KSA), where infection was acquired mainly through horizontal transmission early in life, and less commonly by vertical transmission similar to what is observed in other HBV-endemic countries. (2,5,6,7,8,10,87,103,105) From a historical perspective, the first large-scale community-based epidemiological study conducted on Saudi children showed a hepatitis B surface antigen (HBsAg) seroprevalence of approximately 7% and a > 70% prevalence of at least one HBV marker(4).

Despite significant decline in the prevalence of HBV infection in Saudi Arabia, this viral diseases cause significant morbidity and mortality, and impose a great burden on the country's healthcare system. In 2007, the Saudi Ministry of Health (MOH) ranked viral hepatitis as the second most common viral disease after chickenpox, with almost 9000 new cases diagnosed in that year (52% HBV)(2,82).

Progression from acute to chronic HBV infection is influenced by the patient's age at acquisition of the virus; age is also related to a dichotomy in the clinical expression of HBV infection between high-prevalence (e.g., Asian) and low-prevalence(e.g., Western) countries, where In the Far East, HBV infection is acquired perinatally. In contrast, in the West, most acute HBV infections occur during adolescence and early adulthood because of behaviors and environments that favor the transmission of bloodborne infections, such as sexual activity, injection-drug use, and occupational exposure.

In immunocompetent adults, a strong cellular immune response to "foreign" HBV proteins expressed by hepatocytes results in clinically apparent acute hepatitis, which, in all but approximately 1% of persons infected, affects clearance of the infection(28,53,72).

Macrophage activation represents one of the first events of innate resistance against intracellular infection. In response to pathogens, macrophages and other inflammatory cells secrete cytokines (gamma interferon [IFN-_], interleukin-1 [IL-1], IL-6, IL-8, tumor necrosis factor alpha [TNF-_], and IFN-_). Some of these cytokines lead to activities against pathogens, activate effector cells involved in the cellular interactions that occur during inflammation, and are part of the acute and chronic stages of viral hepatitis (51,84,112). The antibody response in patients with HBV infection plays a critical role in viral clearance through the formation of complexes with viral particles and their removal from the circulation (15,22,75). The specific cellular immune responseplays a main role in the hepatic necrosis that occurs with HBV infection and in the persistence or lack of persistence of viral infection. Certain cytokines can contribute to this process by efficiently inhibiting viral replication when the subtype Th1 cytokine secretion pattern is predominant or by facilitating the propagation of the pathogens in the patient if the subtype Th2 cytokine secretion pattern is predominant (64).

Studies carried out with cultures of peripheral blood mononuclear cells from patients with acute HBV infection showed a Th1-like cytokine pattern with increased levels of production of IFN-_ and TNF-_ (9,15). This high level of cytokine production stimulates the immune response, allowing the cure of HBV disease (45). On the other hand, decreases inthe levels of IL-2 and TNF-_ synthesis and increases in the levels of IL-1 and the soluble form of the IL-2 receptor (sIL-2R) in serum have beenobserved in patients with chronic HBV infection (83), while high levels of IL-4 and IL-6 were found in patients with autoimmune chronic hepatitis (9). Recent studies have demonstrated that clones of T cells canbe characterized by the expression of CD30, which is a member of the TNF family (35) expressed in Th2 CD4_ T cells. Its soluble form (sCD30) is liberated by proteolysis from the outside 105-kDa portion of T cells following cellular activation (25). High serum sCD30 concentrations have been detected in patients with active illness in whom a Th2-type immune response is dominant, like patents with chronic hepatitis due toHBV (37,84).

HBV replicates *via* the reverse transcriptase enzyme system which lacks proofreading ability; therefore, new virions possess diverse genetic variability (50). Different election pressures such as host immunity (endogenous pressure), and vaccine or antiviral agents (exogenous pressure) influence the production of HBV quasispecies in infected individuals. It has been demonstrated that mutations in the HBV genomenot only impact the replication fitness of the virus (phenotypical effect) but can also influence the disease outcome, as well as the response to treatment (clinical effect) (59). Mutations in the HBV surface (S), precore (PC) and basal core promoter (BCP) genes are observed frequently in HBV infected patients, and studies show that these mutations are associated with the clinical outcomes of HBV disease (21,60). The most clinically relevant mutations in the S region arise in the immunologic "a determinant" domain and neutralizing antibodies (anti-HBs) are targeted against this epitope (56,67). The basic core promoter (BCP, nt 1742–1849) and its adjacent precore (preC) region are crucial for replication of HBV. BCP binds various liver factors and preC forms ε structure in pregenomic RNA (pgRNA) as the encapsidation signal (102). Changes in viral replication may influence the progression of liver diseases, particularly in fulminant hepatitis and acute exacerbation of chronic hepatitis (3,20,56,113). Mounting evidence has emerged to demonstrate that BCP and preC mutants are predisposed to severe and progressive liver diseases after HBV infection, causing an increased risk for hepatocellular carcinoma (HCC) (36,110,,127,129). For instance, mutations T1762/A1764 and A1899 have been reported to be independent risk factors for HCC (19), and T1653 and/or V1753 mutations are believed to promote the process of liver degradation (109). However, the association of these mutations with severe symptoms is manifested in certain populations but not in others (1,3,20,98,125).

For acute infection, no medication is available; treatment is supportive. For chronic infection, several antiviral drugs (adefovirdipivoxil, interferon alfa-2b, pegylated interferon alfa-2a, lamivudine, entecavir, and telbivudine) are available. Persons with chronic HBV infection require medical evaluation and regular monitoring to determine whether disease is progressing and to identify liver damage or hepatocellular carcinoma(119).

The majority of the medications now in use for hepatitis B treatment were approved by the Food and Drug Administration (FDA) in 2002 or later; two forms of alfa 2 interferon and five oral nucleoside/nucleotide analogues have been approved, and other medications are in clinical trials(119).

For long-term protection against HBV, there are two types of vaccines: plasma-derived HB surface antigen (HBsAg) vaccine, and yeast-derived HBsAg vaccine(16) HB immunisation, using either type of

vaccine, has been shown to eliminate HBV transmission and prevent HBV-related chronic liver disease(62).

HBV vaccine can be routinely given to children and individuals at risk, along with other commonly used vaccines in a variety of schedules that results in excellent immunogenicity and do not interfere with the immunogenicity of other vaccines (42). The sero-conversion rate aftervaccination is influenced by a number of factors, the most important ones being age and sex. Rates in excess of 95% are seen in young women, whereas the rate may drop to 80% in older men. Immunosuppressed patients, smokers, and obese individuals show even lower rates (100).

The hepatitis B immunization schedule for newborns consists of threedoses (0, 1, 6 month schedule). Booster doses of Hepatitis B vaccine are recommended only in certain circumstances: For **hemodialysis patients**, the need for booster doses should be assessed by annual testing for antibody to Hepatitis B surface antigen (anti-HBs). A booster dose should be administered when anti-HBs levels decline to <10 mIU/mL, and for**other immunocompromised persons** (e.g., HIV-infected persons, hematopoietic stem-cell transplant recipients, and persons receiving chemotherapy), the need for booster doses has not been determined. When anti-HBs levels decline to <10 mIU/mL, annual anti-HBs testing and booster doses should be considered for those with an ongoing risk for exposure. Meanwhile,for persons with normal immune status who have been vaccinated, booster doses are not recommended (119).

Besides, the program includes the prevention of perinatal infection, through pre-maternal screening and prophylaxis of newborns, HBV vaccination for all children, to prevent the infection in childhood and older, vaccination of adolescents who were not protected and individuals belonging to risk groups(38,81).

Literature review:

Hepatitis B is one of the world's most serious infectious diseases. It is estimated that over 350 million people worldwide are chronic hepatitis B virus (HBV) carriers(11,18) Studies show that of all infected persons, 25% have acute hepatitis with jaundice, and 6% to 10% progress to chronic hepatitis(76,88). Between 35% and 40% of all HBV infections diagnosed worldwide every year result from vertically transmitted cases. The risk of infecting their children is increased among women found seropositive for both hepatitis B surface antigen (HBsAg) and precore antigen (HBeAg), an indicator of high HBV titers(93). In an attempt to reduce the spread of this virus, in 1991, the WHO recommended the introduction of HBV vaccination into the Programme of Immunization in all countries(17).

The prevalence of hepatitis B is variable around the world(33). It is greater in high population density areas, such as south-east Asia and sub-Saharan Africa, and in isolated areas, such as Alaska, the Amazon, and some islands of the Pacific Ocean(23,31,104).

In 2004 the World Heath Organization (WHO) estimated that 2 billion individuals, or approximately one-third of the global population, had beeninfected with hepatitis B virus (123). Between 500,000 and 700,000 deaths due to hepatitis B virus infections are estimated to occur each year (43,123), most of which are a result of chronic infection acquired in childhood via maternal or child-to-child transmission (43,129). Sustained reductions in hepatitis B seroprevalence and hepatitis B-related deaths have been observed in countries where universal infant vaccination against hepatitis B is in place. The benefits of infant vaccination are most striking in countries previously of high hepatitis B endemicity (99,129).

Currently, booster vaccination of healthy individuals after priming against hepatitis B virus in infants is not routinely recommended (34,99,114,123). This is based on observations that anamnestic responses are observed in the majority of individuals after exposure to hepatitis B virus infection many years after priming, even in the absence of detectable antibodies at the time of exposure (114). However, the length of long term follow-up studies of hepatitis B vaccination is currently limited to around two decades. Recent studies conducted in individuals primed with plasma-derived vaccines suggest immune memory may begin to wane during the second decade (74,106,118).Two recently published long term follow-up studies of adolescents vaccinated with a recombinant vaccine in infants also suggest waning immune memory over time, but subjects received sub-optimal doses of a vaccine no longer licensed for use (14,47). EngerixTM-B (GlaxoSmithKline Biologicals, Rixensart, Belgium) is a recombinant DNA vaccine containing HBsAg from genetically engineered yeast (*Saccharomyces cerevisiae*). The vaccine has demonstrated protective efficacy of more than 95% in preventing chronic infection with hepatitis B up to 8 years after immunization of children born to hepatitis B carrier mothers (58,90,91,92). This study assessed long term antibody persistence and immune memory 18 years after primary vaccination during infancy with hepatitis B vaccine (EngerixTM-B) (94,95).

Ayoola reported a decrease in the carriage rate from 8.8% before introducing vaccination to 0.9% after it in a hyperendemic region in south western Saudi Arabia in children aged less than 1 year(12).Another report from Taiwan showed that among 1200 children who had received HBV vaccination in infancy, protective antibodies could be found in 71.1% at age 7 and 37.4% at age 12(66).

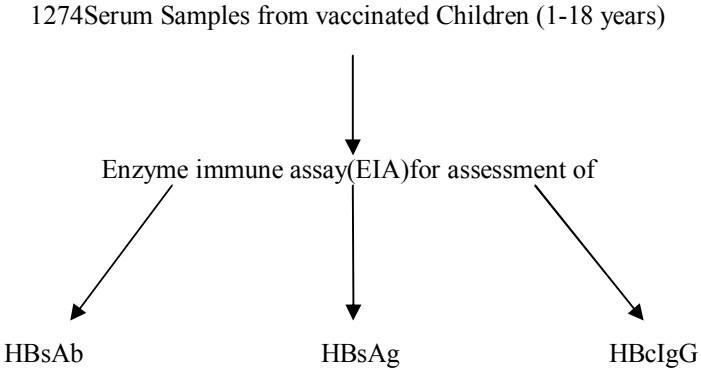
Objectives:

1-To evaluate the immune response to HBV vaccine among vaccinated children with the three doses of HBV vaccine by measuring the level of circulating anti-HB surface antibodies.

2-To evaluate the long-term efficacy of hepatitis B virus immunization program among HBV vaccinated children in Jeddah in preventing hepatitis B virus "HBV" infection by measuring HBsAg,HBcIgG

3- To monitor the possibility of mutant HBV strains in the community, causing failure of vaccination programme.

2.Experimental design



Study subject:

One thousand two hundred and seventy four children were enrolled in this study along a period of 28 months on children age range 1-18 years,724(56.8%) males and 550(43.2%) females,946(74.3%) Saudi, and 382(25.7 %) non-Saudi children were admitted and attended to the children wards of King Abdulaziz University Hospital,(Government) 324(25.4%),and The Children and childbirth Hospital- MOH (Government) 950(74.6%) in Jeddah City, from January 2011 to May 2013 on a random basis. Subjects consent was reported for medical investigation andapproval of hospital director,lab director for using equipments, and lab facilities. Antibody response to Hepatitis B virus vaccine was investigated. Children were chosen based on the following: **Inclusion criteria:** Immune competency, HBV Vaccinated children Age: ranged from one to eighteen years, Sex: males and females**. Exclusion criteria:** Immunocompromised children by blood disorders, congenital and acquired immunodeficiency, and also non-vaccinated children. Data for each child was collected on a precoded questionnaire recording name, age, sex, nationality, admission reasons. Those children whose age below twelve months were excluded.

Reagents and Methods:

Reagents for immunoassay to detect HBsAg in patientssera (BIO-RAD, France) include: microplate coated with human monoclonal antibodies to surface antigen, Conjugate: mouse-anti human Anti-HBs and goat anti-mouse polyclonal antibodies bound to the peroxidase. Reagents for immunoassay to detect anti-Anti-HBs in patients sera (BIO-RAD, France) include: microplate coated with HBsAg (human, ad,and ay subtypes), conjugate: HBsAg (human, ad and ay subtypes) labeled with peroxidase. Reagents for immunoassay to detect anti-HBc antibodies IgG in patients sera (BIORAD, France) include: microplate coated with recombinant core Ag, Conjugate: Goat anti-human IgG peroxidase labeled. Serum specimens were collected aseptically and stored at -20°c till use. Enzyme Immuno assays was performed according to manufacturer manual in the automated EIA machine-BEP III-Siemens.

3.Results:

A total of 1274 serum samples were collected from December 2011 to January 2013,and were tested; 1274/1274(100 %) of these samples were found seronegative for HBsAgand were considered as non infected children.

44/1274(3.5 %) of these samples were found seropositive for HBcIgG, while 1224/1274(96.1 %) of these sampleswere found seronegative for HBcIgG.

340/828(41.1 %) of these samples were found seropositive for HBsAb, while 488/828(58.9%) were found seronegative for HBsAb.Overall vaccinate dresponders, who were showing seropositivity with one marker only HBsAb, were 324/828 (39.1%). Meanwhile, 482/828 (58.21%) were showing seronegativity with all three markers.

4. Discussion:

The protection provided by hepatitis B (HB) vaccine has been well documented (77,78). Antibody

to hepatitis B surface antigen (anti-HBs) concentrations 10 mIU/ml are generally considered protective against hepatitis B virus (HBV) infection (52,77). However, the protective antibodies induced byHB vaccination wane gradually over time and mayreach very low or even undetectable levels (26,116).

Surprising result was, only324/828 (39.1%) who were showing seropositivity with HBsAb.This finding was not matching with those studies reported by Lio*et al.*(70), El-Sawy and Mohamed (32), Hsu *et al.*(55), and Karaglu(57). The efficacy of hepatitis B vaccine of previous studies were 92%, 93.3%, 94.1% and96.7%, respectively. Whereas the vaccine efficacy in other studies which were conducted by Wildgrub*et al.*(124), Pongpithead and Assateerwait(89),Lin and Ou-xang(69), Kuhil*et al.*(61) were 85%, 88.2%, 85.4% and 85%;respectively. Studies of Yvonne *et al.*(128), LiL*et al.*(65), Nedelcu*et al.*(85),XuH*et al.*(126) and Garcia *et al.*(44)showed efficacy of 78.1%, 79.2%,66.3%, 65.8% and 70.6%, respectively(30).

In agreement with our study Lin *et al.*(68)**,** found that the percentage of protective anti-HBs antibody in vaccinated children gradually decreased from 71.1% in 7 years to 37.4% in 12 years old.

Another study was conducted in Senegal and Cameroon in 2005, to assess the HBV immune protection among children. All consecutive children under 4 years old between May 2009 and May 2010, with an immunization card and a complete HBV vaccination, were tested for anti-HBs and anti-HBc. A total of 242 anti-HBc-negative children (128 in Cameroon and 114 in Senegal) were considered in the analysis. The immune response of children with anti-HBs ≥10 IU/L was higher in Cameroon with 92% (95% CI: 87%–97%) compared to Senegal with 58% (95% CI: 49%–67%), (p<0.001). The response to vaccination in Senegal was lower in 2006–2007 (43%) than in 2008–2009 (65%), Several possible explanations may account for these results. First, there may exist problems with storage conditions and compromise the cold chain in Senegal. Second explanation may be related to children's nutritional status (97,117,122).The antibody levels of vaccinated individuals tends to wane duringthe years after vaccination. It has been estimated that 13% to 60%of initial responders lose their detectable antibodies against HBsAg(aHBs) (97,108,115).According to a recent report from Taiwan,15 years after successful vaccination, 30% of the vaccinated children had no detectable aHBs levels. In 33% of the vaccinated children, anti-HB core was detectable, and 1 child had detectable levels of HBsAg.

(18,97). A report from Alaska described a study of 841 patients,of which 84% were successfully vaccinated. During the years after vaccination, 16 patients were found to be infected with HBV (of which 6 tested positive for HBV DNA in serum)(79). Anotherstudy followed 635 successfully vaccinated patients for 5 years (a

total of 773 patients were vaccinated). Of these 635, 27% lost theirmeasurable aHBs, where 55 patients were infected by HBV and 8of these were clinically important (characterized by elevation ofliver enzymes and detection of HBsAg in serum) (46).

Some studies have suggested that lower immune response among Aboriginal infants is genetically determined (41,48,49). Additionally, HBV vaccine escape mutants can lead to vaccine failure(39,120). For practicalreasons, neither HLA typing nor the presence of escape mutants could be assessed in this study setting. Theoretically, lack of immunologicalmaturity in Low Birth Weight (LBW)infants could compromise vaccineresponse; however, when infection and other co-morbid illnesses are excluded, there appears to be no difference (13). It is possible that prematurity, recurrent infection and ongoing poor nutrition in infants contribute to a suboptimal immune response. Recent studies have suggested that immune responses to the early HBV vaccines may have been suboptimal in some Aboriginal communities(41,48,49,121). In addition to factors related to the vaccination process, investigators have suggested that genetic, developmental, and environmental factors may contributeto a poor response(41,48,49). Studies in Mongolia and Indonesia have shown that improper storage and interrupted vaccine transport to remote settings can lead to freezing; this structurally destabilises the vaccine and reduces efficacy(29,86). A study in the Northern Territory, Australia, in 1994documented freezing temperatures in 47.5% of vaccines, either in transfer or during storage(80). In rural China it was thought that similar transport factors could play a part, however it was found that genetic factors played a larger role, with a specific HLA haplotype predicting poor vaccine responses among the Han Chinese (27,96).

The results obtained from previous studies support the notion that theefficacy of hepatitis B vaccine is variable from one study to another and the decline of hepatitis B vaccine efficacy could be attributed to many reasons including:decrease of anti-HBs antibodies titer with increasing age (101), or it may be due to the variability in vaccine synthesis or preparation, defect in vaccine cold chainand differences in the methods used to evaluate antibody titer.Moreover in this study,non-responders subjects 482/828(58.21%) who were showing seronegativity with all three markers, might express either failure of vaccination or immune tolerance, and their immunity

against HBV was absent. The mechanism behind immune tolerance has not been fully elucidated, but HBV-specific T-cell hyporesponsiveness may be partly due to ineffective antigen processing and transport to major histocompatibility complex class I molecules(107).

This phase is mostly seen in patients infected at birth or during early childhood as often seen in Asia(54,111).

Infected children do not mount effective immune responses and exhibit immune tolerance, which leads to a high risk of chronicity in adulthood(54).

However, Kuhil*et al.*(61) and Dahifer (24) who found that the percentages of non responders were 14.6% and 15.6%.Those non-responders children needed extensive investigations to determine the real cause.

Additionally, 1274/1274(100 %) of these children, were found seronegative for HBsAg, which indicates that immunization has a significant impact on hepatitis B virus transmission.

Conclusions and recommendations

Based upon the present study,a serious alarm should paid and attention by the health authorities.Hepatitis B vaccine efficacy was not very high (41.1 %), and HBs Ag was not detected in any participant in the study, this indicates that immunization has a significant impact on hepatitis B virus transmission, therefore we recommend the continuation of hepatitis B universal immunization program and increase the vaccine coverage in Jeddah to 100%, also we emphasize giving the first dose of hepatitis B vaccine immediately after birth and ensure cold chain preservation. Based on the findings of our study there is a trend of decreasing antibody level, we recommend monitoring test of anti-HBs antibody for children to identify those who need a booster dose.

Finally future studies should be conducted to cover other groups and inparticular who are exposed to occupational hazards and studies evaluating other vaccination programs also should be conducted to exploreshortcomings.

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