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PREVENTION AND VACCINATION AGAINST HEPATITIS B VIRUS INFECTION: A REVIEW

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Abstract: Hepatitis B virus, one of the world's most widely spread infectious viral agents, is a major cause of millions of disease and death each year. The existence of approximately 350 million carriers in the world provides a reservoir for continuous transmission of the virus. At present, there is no effective treatment for the diseases caused by hepatitis B virus, and hence prevention of the infection through vaccination plays a significant role in eradicating the diseases caused by the virus. The present review is based on the prevention and vaccination strategies against hepatitis B virus infection and the aim of the study is mainly directed to the development and safety of different hepatitis B vaccines that are already developed and/or on the way to development. The review is mainly based on secondary publications for the last ten years. The review helps in developing ideas about the prevention and vaccination strategies against hepatitis B virus and also the problems that have arisen during vaccination.

Key words: Hepatitis B; Surface antigen; Infection; Vaccine; Prevention; Immunization

Introduction

Viral hepatitis or hepatitis caused by viral agents continue to be a disease of major significance in the world today, both in terms of morbidity and mortality, and in the enormous demands it places on economic and medical resources. Many viral agents are already recognized that are specifically involved in hepatitis of human body. These are Hepatitis A, B, C, D and E (i.e. HAV, HBV, HCV, HDV and HEV). Other viruses like Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Yellow Fever Virus (YFV) etc. can also cause hepatitis. Among these, the five viruses (i.e. hepatitis A, B, C, D and E) have a particular affinity for the liver. Unless otherwise specified, the term "viral hepatitis" is reserved for infection of the liver caused by them (Crawford, 1994; Abbot Diagnostic, 1994).

Of these hepatotropic viruses, both epidemiologically and etiologically, it has been proved that the most infectious and versatile is Hepatitis B Virus (HBV), which is a hardy DNA virus that can withstand extremes of temperature and humidity and can produce

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acute hepatitis, chronic non-progressive hepatitis, progressive chronic disease ending in cirrhosis, fulminant hepatitis with massive liver necrosis and an asymptomatic carrier state with or without progressive disease. Furthermore, HBV plays an important role in the development of hepatocellular carcinoma and provides the backdrop for defective delta hepatitis virus (HDV) (Crawford, 1994).

Globally, liver disease caused by HBV is an enormous problem, with an estimated worldwide carrier rate of 350 million (Grob *et al.*, 1998) and in the United States alone, there are 300,000 new infections per year (Crawford, 1994). Epidemiological data on the prevalence of HBV carriers in the population and the incidence of new infections are used to describe the burden of disease from HB infection. Three epidemiological patterns, each giving rise to different geographical risk areas around the world, have been defined: low (where less than 2% of the population show Hepatitis B surface antigen or HBsAg markers), intermediate (2-8%) and high ($\geq 8\%$) (Grob *et al.*, 1998).

Northern European countries such as Scandinavia, Ireland and the UK and also the North American and Western European countries report that less than 0.1% of the population is HBsAg positive; annual incidence rates of notified cases too are very low, being less than 1/100,000 in the general population. In Southern Europe prevalence rates range from 0.5 to 2%, and incidence rates of notified cases rise to about 6/100,000. The picture is very different in Central and Eastern Europe and in the Newly Independent States (NIS): the virus is highly endemic in some Eastern European countries and parts of the Newly Independent States (NIS). Carrier rates reach a high of $>8\%$ in the Central Asian Republics and in some countries in Eastern Europe, with reported incidence rates varying from 27/100,000 in Kazakhstan to 400/100,000 Turkmenistan. In Africa and China, it has been observed that the carrier rates exceed over 10%. These two areas may be the highest endemic areas of HBV in the world. The countries of Indian subcontinent or south Asia have fallen in the intermediate endemicity area with an HBsAg prevalence of around 5% (Yao, 1996; Yao, 1996; Gill, 1996; Grob *et al.*, 1998).

At present, there is no effective treatment method or system for curing the diseases associated with hepatitis B virus infection. So, till now prevention is the only way of controlling the infection and incidence of this dangerous virus. The objective of this review was to make an elaborate discussion about the prevention strategies especially through the development of safe, efficacious and cost-effective vaccines against the infectious hepatitis B virus. The major informations that are included here are mainly directed to the various types of vaccines of HBV, their efficiency or immunogenicity and safety.

Prevention and Vaccination against Hepatitis B Virus Infection

Considering the severity, transmitting ability and disease producing ability of the versatile hepatitis B virus, a number of control measures have been used to interrupt HBV and reduce the incidence of hepatitis B virus infection in many countries of the world. These include:

proper selection and screening of blood donors for HBsAg and anti-HBc, education of healthcare workers and other high risk groups concerning patterns of transmission, and the use of simple environmental procedures to limit the risk of infection, administration of high titered hepatitis B immune globulin (HBIG/HBIG) when indicated, often in conjunction with vaccines, and vaccination (Hollinger, 1996).

Screening of Blood Donors for Hepatitis B Virus Prior to Blood Collection and Transfusion: The safety of blood and blood products is a global issue. Blood transfusion is an important part of modern medicine, but it is also an efficient route of transmission for a number of infectious agents like HIV and HBV. Although many countries screen all blood donations for a number of infectious agents, a significant proportion of the world's blood supply is either unscreened or poorly screened, with the resultant risk to recipients of transfusion transmitted infections (TTI). Hepatitis B virus (HBV) is of concern because it is transmitted parenterally, many HBV infections are asymptomatic (and infected individuals may thus unwittingly be present as blood donors) and the virus is stable in blood and blood products over long periods. The transmission of HBV can be minimized by screening donors prior to donation, exclusion of high-risk donors, followed by the in-vitro screening for HBsAg (plus anti-HBc in some countries) prior to transfusion (Kitchen, 1998). The establishment of an all volunteers donor population who donate periodically, combined with a stringent health screening process and mandatory testing of blood donors for HBsAg and anti-HBc, has led to the substantial reduction in transfusion-associated hepatitis B. Between 1981-1988, the number of post transfusion HB cases reported to the American Red Cross declined by approximately 50%. At present, HBV accounts for fewer than 10% of the cases of post transfusion hepatitis seen in the United States. The current risk of acquiring an infection is reported to be only 1 in 200,000 for each unit of blood or blood component administered. In most cases sensitivity of HBsAg assay has been the limiting factor in eliminating HBV infection (Hollinger, 1996; Kitchen, 1998).

Precautions and Education of Health Care Workers and other high risks Groups about Hepatitis B Virus Infection: Acquiring HBV is a well-recognized risk among those who work in blood-contaminated environments (such as hemodialysis units, obstetric services, gastrointestinal endoscopy suites, hematology and oncology wards). To lessen the opportunity for infection, healthcare personnel should presume that all body fluids and secretions from HBsAg positive patients are capable of transmitting HBV and should exercise due caution when handling them. Even surfaces contaminated with virus pose a problem because it has been shown that HBV remains infectious after drying and storage at 25 °C for at least 1 week (Hollinger, 1996).

The highest concentration of the virus is found in blood and liver, and the most efficient route of transmission is percutaneous (through broken skin, as by a needle stick). Infection also can occur after direct mucous membrane contact, after oral ingestion of blood, or through contamination of skin lesions, open cuts, or abrasions. Larger doses of virus are apparently necessary to initiate infection via the oral route. Infections after fecal contamination have not been reported. Indeed, virus particles and HBsAg appear to be degraded when added to fecal suspensions or to homogenates of intestinal mucosa. Other

modes of transmission that have not been associated with HBV out breaks include food borne, water borne or air borne routes or via causal contact (Andjaparidze, 1996; Hollinger, 1996).

Prevention of the occupational HBV infection in health care workers has evolved over two decades into four approaches. Two of these – universal precautions and HB vaccine – are the cornerstones of such efforts and at present these approaches are mainly followed, while the other two – patient screening and post-exposure prophylaxis – are of minor importance. The major issues in universal precautions that are adopted for health care workers and other high risk groups include (a) the recognition that all blood and blood-derived body secretions should be considered potentially infectious, and that barriers such as gloves, gowns, masks and protective eyewear should be used where appropriate to prevent direct exposure to blood (b) availability of safe methods to dispose off needles and sharp instruments, and (c) medical education; special training courses to health care workers, on how to handle potentially infectious materials containing hepatitis B virus, e.g. blood, blood products, body fluids and organs. All level hospital workers should have adequate knowledge of the prevention of hepatitis B and other blood-borne infections. Laboratory safety measures such as avoiding mouth pipetting, not eating and smoking while working, rigorous maintenance of a clean environment (free of blood contamination), and emphasizing the importance of handwashing must be adhered to (Andjaparidze, 1996).

Immune-Globulin Immunoprophylaxis (Passive Immunization): Data have clearly established that protection against reinfection with HBV resides with anti-HBs fraction. Lander *et al.* showed that patients who developed anti-HBs failed to contract hepatitis after subsequent rechallenge with HBV (Hollinger, 1996).

a. Pre-exposure Immunoprophylaxis with Immune-Globulin: An experiment in 1974 reported that a conventional immunoglobulin preparation with a low concentration of anti-HBs (1:16 by passive haemagglutination) and a special lot of high titered (1:100,000) human hepatitis B immunoglobulin, designated HBIG, significantly reduced the frequency of HB infections among institutional children when compared with an untreated group. HBIG is similar to conventional IG except that it is prepared from plasma preselected for a high titer of anti-HBs. In the United States, HBIG has an anti-HBs titer of 1:100,000 or greater than RIA. The HBIG appeared to protect by passive immunization, whereas passive-active immunization was more frequently observed in those who received conventional immunoglobulin. Persistent antigenemia was not observed in either the HBIG group or the conventional IG group, but it occurred in 14% of those for whom no treatment was provided (Zuckerman *et al.*, 1995; Abbot Diagnostic, 1994).

b. Post-exposure Immunoprophylaxis with Immune-Globulin: With the availability of a vaccine for hepatitis B and mandatory screening of blood donors for HBsAg and anti-HBc, there is little justification for the use of HBIG in pre-exposure prophylaxis. However, there are situations in which post-exposure prophylaxis is essential or desirable. The most common situations requiring immunoprophylaxis with HBIG (usually in conjunction with vaccination) are:

exposure of an individual to HBV containing material by percutaneous inoculation, oral ingestion, or direct mucous membrane contact, transient HBV contact through sexual exposure by the partner(s) of an acutely infected patient, and fetal/neonatal exposure to mothers with HB (Hollinger, 1996).

Timing of the initial dose of IG after exposure and the concentration of HBV present in the source material are important factors in determining how successful prophylaxis will be after the administration of anti-HBs. For isolated episodes, effectiveness appear to diminish rapidly if administration is delayed for more than three days. Most of the regimens used today combine passive immunization with active immunization induced by vaccine, thereby providing immediate protection with more durable immunity (Kar, 1996; Zuckerman *et al.*, 1996; Hollinger, 1996).

Three needle-stick studies, one uncontrolled, were conducted under circumstances that permitted a comparison of HBIG with immune globulin containing levels of anti-HBs. The uncontrolled trial by Krugman *et al.* favored the administration of HBIG over conventional IG through passive-active immunization. In this study, HBV was intentionally administered to residents of a custodial institution 4 hours before inoculation with HBIG or IG. The HBV dose was administered via a needle stick or during sexual exposure. Subsequent investigation showed that the antibodies in the immune globulin lot were directed against a different subtype than the HBV used to infect the children. In addition, the immune globulin preparation contained a very low concentration of anti-HBs as compared with current lots of IG. Based on the high secondary attack rates that occur among sexual partners of persons with acute hepatitis B, the administration of IG to spouses or sexual consorts seem justified. (Gitlin, 1997; Kar, 1996; Zuckerman *et al.*, 1996)

Redeker *et al.* compared HBIG with an anti-HBs free IG placebo. Despite a sevenfold reduction in the incidence of HB observed in the HBIG group (4% versus 27%; 85% efficacy), the study has remained controversial. Major concerns have focused on the fact that:

- i. some of the participants were intravenous drug abusers (more than 10% of the exposed sexual partners were excluded subsequently because their initial blood specimens contained HBsAg);
- ii. the attrition rate was high, and
- iii. IG of unknown composition had been given to some of the volunteers by their primary physician before entry into the study.

In an attempt to resolve some of these issues, Perritto *et al.* randomized 40 spouses or sexual consorts of patients with acute HB and gave them single doses of HBIG or low-dose IG. Unlike the study by Redeker *et al.*, no placebo group was evaluated. So, protective efficacy could not be ascertained. Only one clinical case of HB occurred in the low-dose IG group- implying that both IG provided protection, but the lack of a placebo group complicates this conclusion. Subclinical infections occurred on three of 21 volunteers in the low dose IG group, compared with two of 19 on the HBIG group (Kar, 1996; Zuckerman *et al.*, 1996; Hollinger, 1996).

Maternal-neonatal transmission of HBV presents special problem in prophylaxis. Prevention of chronic infection is the goal because most infected infants are not overtly ill. The development of chronic HB in such infants is especially alarming in view of its eventual associated with chronic liver disease and hepatitis B chronic carrier (HCC) in some adults. In a double blind, controlled trial in Taiwan, a small single dose of HBIG was given within 7 days of birth to infants of HBsAg carrier mothers. Except for prolongation of the incubation period in those given HBIG, no overall differences in the rate of chronic infection were found between HBIG treated infants and the control group. However, HBIG may have reduced the incidence of chronic antigenemia when it is given within 48 hrs of birth. In two uncontrolled studies large doses of HBIG given in multiple doses to neonates of HBsAg positive mothers were protective. In the first study, none of six high risk infants (versus four of six untreated infants) became carriers after receiving 0.5 ml/kg every months for 6 months. Forty three infants in the second study received 0.16 ml/kg of HBIG immediately after birth, then approximately every 4 months for 1 year, and none became chronically infected. (Hollinger, 1996)

Development of established Hepatitis B Vaccines:

a. Plasma derived Vaccine: Active immunization against HBV in humans was initially investigated using a crude immunogenic preparation of HBV containing serum (MS-2 strain; subtype ayw; titer of $10^{7.5}$ chimpanzees infectious doses per milliliter). The serum was diluted 1:10 and heat inactivated at 98°C for 1 minute. The heat-treated inoculum did not result in clinical or biochemical evidence of hepatitis in 29 vaccinated children, although anti-HBc seroconversion occurred in a few children. Between 4 and 8 months after receiving one to three inoculations, the group was challenged with the unheated HBV containing serum. This was followed by complete protection 17 (59%) and transient, subclinical infection in three (10%). These studies led to the development of more sophisticated HBV vaccines harvested from the plasma of healthy human carriers. When tested in chimpanzees, these subvirion vaccines were found to be safe and efficacious. Efforts were initially directed toward ensuring that the plasma-derived vaccine was free from nucleic acid and, thus non-infectious. These vaccines consist of highly purified, formalin inactivated and/or heat-inactivated, alum absorbed, HB subvirion particles (22nm) of HBsAg that are free of detectable nucleic acid. The antigen is harvested from the plasma of asymptomatic, apparently healthy human carriers of HBV by a series of steps that may include precipitation, ultra-centrifugation, gel filtration, and/or affinity chromatography. Both monovalent and bivalent subtype vaccines have been produced. In at least one vaccine product, the starting material came from HBsAg/anti-HBc positive donors. (Grotto *et al.*, 1998; Gill, 1996; Hollinger, 1996)

b. Recombinant Yeast Derived Vaccine: Yeast derived recombinant vaccines were developed initially to counterbalance some of the perceived but unfounded, concerns regarding the safety of plasma derived vaccine. However from the manufacturers viewpoint, in fact that the production cycle could be decreased from 65 weeks to 12 weeks, batch-to-batch consistency could be more easily attained, and a continuous supply of material could be generated made the new technology very attractive. Thus, the licensure of a recombinant DNA derived vaccine in 1986 rendered the plasma derived vaccine obsolete in the U.S.A

and also in the rest of the world even though it contains superior immunogen. (Grotto *et al.* 1998; Hollinger, 1996).

Current US-licensed yeast-derived vaccines of HBsAg are produced in large fermentation cultures of *Saccharomyces cerevisiae* yeast cells. This yeast carry a recombinant yeast vector (plasmid) consisting of yeast specific promoter sequence, a yeast gene specifying a glycolytic enzyme, the s gene, and plasmid DNA from *E. coli*. HBsAg is released from the cells by homogenisation or disruption with glass beads, is purified by clarification, and is then absorbed onto aluminium hydroxide, after which thiomerosal is added as a preservative. The two major yeast derived HB vaccines that are licensed in most countries are Engerix-B[®] (SmithKline Beecham, Philadelphia, PA) and Recombivax-HB[®] (Merck & Co. West Point, PA.). These products are structurally and chemically similar and contain less than 2% yeast protein and no detectable yeast DNA.

i. Major Adverse Effects of Yeast-Derived Hepatitis B Vaccines: In the clinical trials with recombinant HB vaccines, the most frequent side effects reported were injection site soreness sometimes accompanied by erythema (3-29%), fatigue (15%), headache (9%), and temperature greater than 37.7°C (1-6%). Other reported symptoms (such as gastrointestinal upset) seemed temporal and not causal. Review of the post-marketing surveillance literature (4.5 million doses) revealed an overall rate of one adverse effect per 15,500 doses distributed. Of these, local reactions were reported to be the only events unequivocally related to the vaccine (at a rate of 1 in 85,000 doses). These reactions included nausea, rash, headache, fever, malaise, injection site symptoms, fatigue, influenza like symptoms, vomiting, dizziness, pruritus, arthralgia, myalgia, diarrhea, urticaria, paraesthesia and somnolence all of which resolved, generally within 24-48 h of vaccine administration. Reactions were less frequent with subsequent doses. No serious or severe reactions attributable to the vaccines were reported. Since the publication of the above-mentioned surveillance (1990), some major adverse events have been reported. These include immediate reactions (anaphylaxis and urticaria) as well as delayed reactions, including skin, rheumatic, vasculitic (including systemic lupus erythematosus and glomerulonephritis), hematologic, ophthalmologic and neurologic reactions. (Grotto *et al.*, 1998)

ii. Factors Influencing the Immune Response of Yeast Derived Hepatitis B Vaccine:

a. Immunization Factors: Characterizations of the immunization process that influence immunogenicity include dosage, number and timing of inoculations, storage of vaccine, type of adjuvant particles, and the use of methods to enhance antibody response.

Dosage and Immunization Schedules: Several studies have shown unequivocally that the dose of vaccine administered to a subject is of considerable importance in determining the peak immune response achieved by that person. Data in New Zealand with 2 microgram of plasma derived or recombinant vaccine, administered at three times at monthly intervals to children from 1 to 14 years of age, have demonstrated acceptable responses. Moreover, a number of vaccine schedules have been evaluated, but none has supplanted the current regimens. In contrast, the highest responses occurred when vaccine was give at 0, 1 and 6 (or 0,1 and 12) and 0, 2 and 4 months. Slightly lower responses were observed in subjects given vaccine at 0, 2 and 6 or 0, 2 and 9 months. A

good response to the 0, 2 and 4 or 0, 2 and 6 months schedule is encouraging because these regimens are similar to what is being proposed for the universal immunization of infants. Currently, two primary immunization series are being instituted using three injections at 0,1 and 6 months or four injections at 0, 1, 2 and 12 months. For routine pre exposure prophylaxis, the 0,1 and 6 month schedule is preferred, whereas the four-dose regimens may be advantageous for immunocompromised patients or in post exposure prophylaxis situations. However, the addition of HBIG to the post exposure regimen may regale this advantage. (Safary, 1996; Hollinger, 1996)

Storage of Vaccines: Inadvertent freezing of alum adsorbed, plasma-derived vaccine during a clinical trial indicated that such a practice might be deleterious to immunogenicity. Although no additional clinical trials have been designed to verify this observation, animal studies have confirmed that freezing reduces the immune responses, presumably by altering the physical properties of the adjuvant and its relationship to the immunogen. For this reason, freeze detectors should be included in all vaccine shipments to ensure that freezing does not occur during any step in the transportation or storage process. Conversely, storage of a recombinant derived vaccine at temperatures of 37^oC for up to 4 weeks did not reduce the immunogenicity of the product in a significant manner. Potency is optimally preserved when vaccine is stored at 2 to 8^oC (Hollinger, 1996).

Adjuvant Particles: Adjuvant particles also take a good part in increasing the immune response of a vaccine. Commercially available adjuvant that is used in hepatitis B vaccine preparation is aluminium hydroxide. In a previous study, it was found that a lipophilic derivative of muramyl dipeptide [MDP-Lys(L18)] augmented antibody response to recombinant human hepatitis B surface antigen (rhHBsAg) when it was co-immunized with rhHBsAg solubilized in PBS (Koike *et al.*, 1998).

Recently, Koike *et al.* also examined adjuvant activity of two bacterial cell-derived adjuvants such as Bacillus Calmett Guerin cell wall skeleton (BCG-CWS) and trehalose-6,6-dimycolate (TDM), to enhance immunogenicity of rhHBsAg, comparing their activity with that of MDP-Lys (L18). They found that activity of BCG-CWS and TDM to enhance antibody induction seemed to be almost the same with that of MDP-Lys (L18). Furthermore, the enhanced antibody response raised by these adjuvants was shown to be due to high titers of HB antigen-specific IgG1. In addition, the activity of these three adjuvants to enhance antibody response was shown to be higher than that of the present clinical vaccine, aluminium hydroxide-attached rhHBsAg (rh HBsAg-alum). To enhance the immune response in immunodeficient individuals such as hemodialysis patient, interferon alfa and gamma have been used with moderate success as adjuvants to the conventional vaccines. Better results were achieved in a pilot study by Meuer, *et al.* in which interleukin-2 was injected together with vaccine. However, a large, controlled study by Jungers could not confirm these results. An experimental new adjuvant, monophosphoryl lipid A or MPL, was tested in a clinical trial showing a slightly better result than the conventional vaccine (Jilg, 1998).

Researchers are still trying to develop good quality adjuvants, which can stimulate better immune responses as well as not create any harmful effects.

b. Host Factors: Host factors such as age and weight of the subject, genetics, immunological competency, malnutrition and smoking may influence the immune response to the vaccine.

Age and Weight of the Subject: Increasing age, especially above 30yrs, has been shown to correlate independently with a decreasing response to the vaccine. In one study, anti-HBs seroconversion rates declined from a relatively low frequency of 69% in persons 61 to 70 years of age to less than 39% in those more than 80 years of age. Suboptimal responses occur in obese persons. Correspondingly, the immune response is inversely related to weight or, more precisely, the Quetelet index of body mass, especially in these subjects who are under 30years of age (Hollinger, 1996).

Genetic, Immuno-suppression, Malnutrition and AIDS: White children seemed to respond better to the vaccine than did nonwhite children (Asian, African-American), but the number of subjects was relatively small (Hollinger, 1996). Immunosuppression groups, such as hemodialysis patients and those on chemotherapy or infected with HIV, respond poorly to vaccination when compared with healthy individuals (Tayal and Sankar, 1994).

Immunosuppression due to vitamin A deficiency and protein-caloric malnutrition could also act similarly. Worldwide, millions of children have severe vitamin A deficiency or protein-caloric malnutrition, conditions that adversely affect the immune system. Children tend to have reduced CD4 and CD8 cells. Infants born to mothers on substance abuse could also respond poorly to HB vaccines. Vitamin A deficiency in pregnant females contributes to mother-to-child transmission of HIV. The mean vitamin A concentrations in mothers who transmit HIV to their infants are lower than in those who do not. The performance of HB vaccine in certain areas in Africa, where the vertical HIV transmission ranges between 25% and 50%, could be sub-optimal due to vitamin A deficiency and HIV/AIDS. (Arya, 1995)

c. Current Combined vaccines with Hepatitis B: The availability of safe, efficacious HBsAg vaccines provides public health officials with an opportunity to reduce the incidence of HBV through mass immunization programs. To achieve this goal, the HBV vaccine is being integrated into the Expanded Program on Immunization (EPI) as recommended by WHO. At present, more than 90 countries of the world have included this vaccine in the national immunization programs. Its combination with DTP (diphtheria-tetanus-pertussis) and other childhood vaccines will lower administration cost, simplify vaccine administration and improve the logistics of vaccine delivery. It is believed that the combined vaccines will increase acceptance and vaccine coverage. The immunogenicity and reactogenicity of the various components should not differ when given separately or together. The combined vaccines DTP+HB (diphtheria-tetanus-pertussis plus hepatitis B), DT+HB (diphtheria-tetanus plus hepatitis B), HA+HB (heptitis B plus heaptitis A), HB+Hib (hepatitis B plus *Haemophilus influenza* type b conjugate) have already been

developed and used in several countries and DTP+HB+IPV (diphtheria-tetanus-pertussis plus hepatitis B plus integrated polio vaccine) and DTP+HB+IPV+Hib combined vaccines are currently under development. (Papaevangelou *et al.*, 1995; Papaevangelou *et al.*, 1996; Papaevangelou, 1998; Poovorawan *et al.*, 1994)

d. Alternative HBV Vaccines: Although the current HBsAg vaccines have been shown to be safe and efficacious, a number of manufacturers continue to search for alternative vaccines that may be less expensive to produce and will provide greater immunogenicity especially in immunocompromised hosts. In addition the product should not contain any contaminating proteins and should be free of residual infectious HBV, coexisting viruses, and/or nucleic acid. Some of the proposed vaccines are academic curiosities that are being evaluated for the advancement of the knowledge. Others are of historical interest. Except for the pre S gene products, non-appear likely to replace or supplement the current vaccine at the time.

i. Polypeptide Vaccines: Polypeptide preparations of HBsAg exclude genes of viral or cellular origin and can be readily examined for the presence of host-derived substances. They also offer an excellent opportunity to study the primary sequence or conformational properties of the antigenic determinants required to produce a protective antibody response to HBV. An alum-adsorbed, sodium dodecyl sulfate (SDS) denatured polypeptide preparation of HBsAg was capable of eliciting a long-lived humoral anti-HBs response in four non-immune chimpanzees that were protected from infection after challenge with HBV. More recently, a dimer of p25 and gp30 was isolated from purified 22nm HBsAg particles using a non-ionic detergent under non-reducing conditions. Detergent removal allowed the polypeptides so that the hydrophobic regions are sequestered in the interior, with the hydrophilic residues on the outside. Similar HBsAg polypeptide vaccines packaged in a micellar configurations and alum adsorbed produced superior anti-HBs response in mice, chimpanzees and humans. In addition, the rapid anti-HBs response that occurred in human after the initial inoculation (75% seroconversion rate in 2 weeks) and the superior anti-HBs levels achieved, suggest that such an immunogen might be beneficial in post exposure prophylaxis where the early development of immunity is advantageous (Hollinger, 1996; Zuckerman *et al.*, 1996).

ii. Hepatitis B Vaccines Containing pre-S epitopes: A disadvantage of plasma-derived and recombinant hepatitis B vaccines containing only the major protein of HBsAg (without pre-S sequences) is the lack of immune responsiveness of a minority of vaccines. The identification of an immunodominant domain in the pre-S2 region of HBsAg (Neurah *et al.*, 1985) and the observation that mice which were immunologically non-responsive to the major protein of HBsAg made antibodies to a synthetic peptide corresponding to this epitope (Millich, *et al.*, 1985; Neurah *et al.*, 1985) stimulated interest in incorporation of pre-S sequences in hepatitis B vaccines. Itoh *et al.* (1996) demonstrated that a synthetic peptide encompassing 19 amino acids from the pre-S2 region, when coupled to keyhole limpet haemocyanin, elicited a protective antibody response when administered to chimpanzees (Itoh *et al.*, 1996). The middle (pre-S2+S) and large (pre-S1+pre-S2-S) forms of HBsAg have been expressed in yeast using constitutive and inducible promoters respectively (Ellis *et al.*, 1988). The former preparation has been evaluated for safety and

immunogenicity (Miskovsky, *et al.*, 1991). A vaccine containing all three (large, middle and major) forms of HBsAg, synthesized in Chinese Hamster Ovary (CHO) cells, has been tested in Singapore. The preparation proved safe and immunogenic with a rapid antibody response in 96% of the recipients (Yap *et al.*, 1992).

iii. Use of Antiidiotypes as Vaccines: Antibodies directed to the variable region of another antibody molecules define the unique features or idiotype of the antibody, and such antibodies are called antiidiotype antibodies. Antiidiotypes often are mentioned as potential vaccine candidates against HBV. In one study, two chimpanzees were immunized with an antiidiotype to anti-HBs, developed anti-HBs and were protected from injections with HBV after challenge. Major concerns revolve around the potential for hypersensitivity or anaphylactoid-type reactions resulting from multiple injections of heterologous antibody. However, the potential to prime a non-responder host deserves further study (Hollinger, 1996).

e. Other Genetically Engineered Vaccines:

i. Hybrid (Live) Virus Vaccines: Live virus vaccines should be less costly to produce, easy to administer, and have an extended shelf life. Recently, investigators have constructed potential live vaccine against HBV by inserting HBsAg into vaccinia virus or adenovirus genomes. It is also possible to construct infectious vectors that contain genes from more than one virus, thus providing several immunizations simultaneously. (Hollinger, 1996; Zuckerman *et al.*, 1995). Recently, a recombinant Oka varicella-HBV vaccine was constructed in Japan by inserting the HBsAg gene coding partial PreS2 and whole S regions into the viral thymidine kinase gene. Recombinant virus induced antibody response to both varicella-zoster virus (VZV) and HBsAg in guinea pigs and the level of antibody titers to HBsAg was comparable in guinea pigs immunized with a live varicella-HBV vaccine or commercial HBs subunit vaccine. This recombinant virus may be a good candidate for the live varicella-HBV vaccine (Shiraki *et al.*, 1992; Shiraki *et al.*, 1993). The advantages of recombinant hybrid virus recombinants as vaccines include low costs, ease of administration, vaccine stability, long shelf life, possible use of polyvalent antigens and good candidates for possible combined vaccines (Zuckerman *et al.*, 1995).

ii. Nucleic acid Vaccine; A possible HB vaccine of the future: Nucleic acid vaccines represent a new approach to the control of infectious agents. These novel vaccines are both easy to construct and produce. Recombinant DNA technology is used to clone DNA sequences encoding the protein or proteins to be used as immunogens into a eukaryotic expression vector. The constructed plasmid is grown in bacteria and purified. The purified plasmid DNA is then directly inoculated into the animal to be vaccinated. The expression of the plasmid DNA by cells in the inoculated host produces the protein that raises the immune response (Robinson, 1997). Direct inoculations of vaccine DNAs into animals generate antibody, cytolytic T cell (CTL) and protective immune responses. Quite impressively, nanogram levels of DNA-expressed proteins can raise high immune responses. Initial skepticism about the potential of this new technology reflected the disbelief that such small amounts of protein (100-1000 times less than used for inactivated viral or subunit vaccines) could initiate immune responses. Protective immune responses can be generated by skin, muscle, and intravenous inoculations of DNA. Immunizations can be accomplished by injecting DNA in saline, or by using a gene gun to propel DNA-coated

gold beads into cells. The raised responses have good longevity, with even single doses of DNA having the potential to elicit long lasting CTL and antibody responses. (Robinson, 1997)

DNA-based Immunization of Mammals against HbsAg: In mice, immunization by injection of HBsAg-expressing plasmid DNA results in rapid induction of strong and long lasting humoral and cell mediated immune responses. Intramuscular injection of HBsAg-expressing DNA into the TA muscle of the mouse results in transfection of 1-2% of the muscle fibers which have a normal histological appearance 5 days after DNA injection, but are seen to fragment at about 10 days and to disappear completely by 30 days. This is probably due to attack by HBsAg-specific CTL since similarly transfected fibers are spared in mice with severe combined immunodeficiency. Very strong CTL responses are detected with spleen cells of DNA-immunized mice by 6 days after injection of DNA and these are maintained for at least several months. (Davis *et al.*, 1997).

Recently, another important experiment was conducted where to determine whether DNA based immunization could protect newborn chimpanzees against a challenge infection with HBV. Two chimpanzees were immunized on the day of birth with a plasmid coding for HBsAg. Following challenge with infectious HBV particles at 33 weeks, the two immunized animals developed anamnestic antibody responses; however, neither developed detectable HBsAg or antibody to core protein that are the conventional markers of HB infection. Both these markers appeared in an unimmunized control animal. This experiment concluded that DNA based immunization can induce protective immunity in newborn chimpanzees (Prince, 1997).

Potential for HBV DNA Vaccine for Humans: The results obtained to date in animals indicate that DNA may be better than recombinant protein for immunization against HBsAg with respect to rapidity, strength and longevity of humoral and cellular immune responses. If equally efficacious for humans, DNA vaccines could be useful for prophylactic and/or therapeutic immunization. Combined with the many economical and practical advantages of DNA this would be particularly useful in less developed areas of the world. As a prophylactic vaccine, the DNA approach may be superior to the use of recombinant HBsAg, especially for the induction of a very rapid humoral response in neonates born to chronic carrier mothers¹⁵. In addition, a DNA vaccine may be effective, possibly because of the induction of strong CTL, for therapeutic immunization of HBV chronic carriers. Nevertheless, despite the promising results in animals, it will be desirable to further optimize either the efficiency of the direct gene transfer or the strength of the immune response to the expressed antigen, so that the lowest possible dose can be used on humans. (Davis *et al.*, 1997)

iii. Oral Hepatitis B Vaccines: The two major hydrophilic regions of the hepatitis B virus surface antigen (HBsAg) have been expressed in the outer mannoprotein layer of the cell wall of "Bakers Yeast", *Saccharomyces cerevisiae*, by fusing them between the yeast invertase signal sequence and the yeast alfa-agglutinin carboxyterminal cell wall anchoring sequence. The fusion protein contained most of the pre-S sequences, including

the hepatocyte receptor, and part of the S sequence including the “a” determinant, and was expressed from multiple genomic copies using the *PCK* promoter. Immunofluorescence studies showed that the fusion protein was detectable at the cell surface and was stably expressed at a relatively high level. Intraperitoneal immunization of mice revealed a very weak response against the S region, and a high response against yeast itself. It is proposed that increasing the amount of the antigen and reducing the number of native cell wall proteins might lead to a yeast that is usable as a safe and cheap live oral vaccine (Schreuder *et al.*, 1996).

In another study, recombinant *Salmonella* containing the gene coding for HBsAg was used as an experimental vaccine in mice and induced an anti-HBs response (Schodel *et al.*, 1990). A more futuristic approach, which has been explored, is the development of transgenic plants, such as bananas, which express HBsAg as edible vaccines. These vaccines may be available in the near future. (Mason *et al.*, 1992; Jilg, 1998)

Developing New Hepatitis B Immunization Strategies:

Not only developing highly safe and immunogenic vaccines and establishing effective immunization strategies, but to aware people about the severity and infectivity of this virus might be the true steps in preventing and controlling the virus infection. For that reason, routine screening of blood donors, pregnant women, drug abusers, health care workers, and others high risks groups; and vaccination of high risks babies and other high risks groups, all infants, all adolescents and also to vaccinate everybody must be practiced thoroughly.

There is now strong support for the introduction of universal antenatal screening to identify hepatitis B carrier mothers and the vaccination of their babies. It is important that any other strategies do not interfere with the delivery of vaccine to this group. Immunization of this group will have the greatest impact in reducing the number of new hepatitis B carriers. For children outside this group it is difficult to estimate the lifetime risk of acquiring a hepatitis infection.

New hepatitis B immunization strategies that have developed to control the infection of HBV contain the following four main approaches:

- Continue vaccination of the ‘high-risk’ babies as defined above.
- Vaccinate all infants.
- Vaccinate all adolescents.
- Vaccinate everybody (Zuckerman *et al.*, 1995).

In United States, a similar strategy is strictly maintained which is comprised of the following components:

- Preventing perinatal transmission.
- Routine infant vaccination.
- Catch-up vaccination of children in high-risk groups at any age.
- Catch-up vaccination of all children at 11-12 years of age.
- Vaccination of adolescents and adults in high-risk groups.

By following the above strategy, a great deal of success has already been achieved in US. According to recent surveys, >85% of pregnant women are screened for HBsAg. Of infants born to HBsAg positive mother identified in 1995, 93% received appropriate immunoprophylaxis at birth; however, only 69% were fully vaccinated by 6-8 months of age. From 1991 (when routine infant HB vaccination was first recommended) to 1996, the proportion of 19-35 month old children who have received three doses of HB vaccine has increased from <10 to 83%. During this time, rates of acute HBV infection 7-10 years of age have declined by 27% and rates among children 3-6 years of age have declined by 62% (Mast *et al.*, 1998).

Discussion

Despite the widespread availability of safe and effective HB vaccines for some 15 years, HBV remains a serious public health threat worldwide. It is estimated that globally 2,000 million people have been infected with HBV at some time in their lives, and that 350 million are chronic carriers of HBV (Grob *et al.*, 1998).

In our country, epidemiological study based on hepatitis B virus is still at infancy. It is estimated that around 5-6% people in our country are chronic carrier of HBV. In early 1990s, prevalence of HBsAg in Dhaka city was investigated among three risks groups (professional blood donors, parenteral drug abusers and sex-workers). Results showed that, the sera of 20% blood donors, 11% sex-workers and 8% parenteral drug abusers were positive to HBsAg (Mustafa *et al.*, 1991). This experiment suggested a high prevalence rate of hepatitis B in Bangladesh.

Similar to other developing countries in the world most of the people in our country are little concerned about HBV infection. Moreover, till now no national propaganda has been undertaken to aware people about the infectivity and severity of this infectious virus. EPI (Expanded Program on Immunization) of Bangladesh is yet to adopt hepatitis B vaccine in it's National Immunization Program. But since 1991, when the World Health Organization (WHO) set 1997 as the target for integrating HB vaccine into all immunization programs, more than 90 countries of the world have done so, either as part of infant or adolescent immunization programs.

Although the HB vaccines are available in our country, the prices are beyond the means of common people and only for that reason, even if they are aware about the HBV infection they are unable to accept it. This problem may be the main drawback for preventing the HBV infection in Bangladesh.

It is possible to take effective steps in controlling and preventing the hepatitis B virus infection through distribution of the vaccines from Govt. Hospitals with affordable prices, through integration of the vaccine into National Immunization Program and also through the development of national propaganda to aware people about the transmission, infectivity and severity of this virus. These may be the major concern of our government.

If not properly and immediately taken, hepatitis B virus infection may become a common health problem in the near future.

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