# การเปรียบเทียบภูมิคุ้มกันจากวัคซีนตับอักเสบชนิดที่ผลิตจากยีสต์อย่างเดียวกับวัคซีนร่วมกับ Hepatitis B Immunoglobulin ในเด็กแรกเกิดจากแม่ที่มี HBeAg

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## บทคัดย่อ

ได้ศึกษาการป้องกันการแพร่เชื้อไวรัสตับอักเสบบีจากมารดาสู่ทารก ระหว่างปี พ.ศ .2531-2534 ในทารกที่เกิดจากแม่ที่มี HBsAg/HBeAg ที่โรงพยาบาลทหารเรือกรุงเทพฯ จำนวน 90 ราย โดยการฉีดวัคซีนตับอักเสบบีที่ผลิตจากยีสต์เมื่อแรกเกิด 1 เดือน และ 2 เดือนหลังเกิด แบ่งทารกเป็น 4 กลุ่ม กลุ่ม A ได้รับวัคซีนตับอักเสบบีที่ผลิตจากยีสต์ขนาด 10 ug อย่างเดียว กลุ่ม B ได้รับวัคซีนขนาด 10 ug ร่วมกับ hepatitis B immunoglobulin (HBIG) ตอนแรกเกิด กลุ่ม C ได้รับวัคซีนขนาด 5 ug อย่างเดียว และกลุ่ม D ได้รับวัคซีนขนาด 5 ug ร่วมกับ HBIG ตอนแรกเกิด เฉพาะกลุ่ม C และ D ได้วัคซีนเมื่อเด็กมีอายุครบ 1 ปีด้วย พบว่า ภูมิคุ้มกันในเด็กเมื่ออายุ 4 เดือน เท่ากับร้อยละ 93.3, ร้อยละ 100, ร้อยละ 95.8 และร้อยละ 96.3 ในเด็กทั้ง 4 กลุ่มตามลำดับ ค่าเฉลี่ยมัชฌิมเรขาคณิตของ anti-HBs เท่ากับ 113.6 mIU/ml, 104 mIU/ml, 102.01 mIU/ml และ 160.51 mIU/ml ในเด็กทั้ง 4 กลุ่มตามลำดับ เมื่อเด็กอายุครบ 1 ปี พบว่ามีภูมิคุ้มกันร้อยละ 100 ทั้ง 4 กลุ่ม และมีค่าเฉลี่ยมัชฌิมเรขาคณิตของ anti-HBs ในกลุ่ม A เท่ากับ 168.17 mIU/ml กลุ่ม B เท่ากับ 184.48 mIU/ml กลุ่ม C เท่ากับ 143.62 mIU/ml และกลุ่ม D เท่ากับ 265.40 mIU/ml ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่าง 4 กลุ่ม ไม่พบปฏิกิริยาหรือผลแทรกซ้อนของวัคซีน สรุปได้ว่าวัคซีนที่นำมาใช้ในการศึกษาครั้งนี้เป็นวัคซีนที่ให้ภูมิคุ้มกันสูง และมีความปลอดภัยเมื่อใช้ในเด็กแรกเกิดอย่างเดียวหรือให้ร่วมกับ HBIG และยังพบว่าวัคซีนขนาด 5 ug ให้ผลดีใกล้เคียงกับขนาด 10 ug จึงเห็นควรแนะนำให้ใช้ 5 ug เพื่อลดค่าใช้จ่ายของการให้วัคซีน

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# Introduction

In area fo hyperendemicity such as Asia and Oceania, hepatitis B virus (HBV) is transmitted from asymptomatic carrier mothers to their babies, especially when mothers are seropositive for HBe antigen (Pongpipat at. al., 1980). The risk of perinatal transmission is especially high. Between 65% and 90% of these newborns have been shown to become chronic carriers (Wong at. al., 1984; Beasley at. al., 1983; Xu at. al., 1985 and Pongpipat at. al., 1986). To intervene the mother to baby transmission the plasma derived HB vaccine with HBIG or HBIB alone had already been shown to be immunogenic in Thai meonates (Pongpipat at. al., 1983 and Pojanagaroon, 1988). However the high cost and limited availability of the plasma derived HB vaccine had led researchers to investigate alternative methods. Recently yeast derived vaccine obtained by DNA recombinant technology has been prepared. Previous studies have shown the safety and immunogenicity of one such vaccine in neonates (Poovorawan, 1989).

The aim of the present study is to compare the immunogenicity and safety of the recombinant DNA yeast derived HB vaccine with HBIG and yeast derived HB vaccine alone in healthy newborns of HBsAg/HBeAg positive mothers.

# Materials and Methods

**Serologic status of pregnant women**

Beginning in April 1988, the sera of 7,153 pregnant women who have visited the obsteric clinic at the Royal Navy Hospital, Bangkok, Thailand were tested for HBsAg and HBeAg The HBeAg positive pregnant women were informed of the HB vaccine trial and consent was obtained for treatment of their newborns. To be eligible for the study, the infants had to be in good health at birth. Specifically the infants had to weigh at least 2,000 g and had a five minute Apgar score of 7 or more.

**Recombinant DNA derived HB vaccine**

It was developed by the Chemo-Sero-Therapeutic Research Institute, Japan in 1984. The vaccine is composea of only 5 gene product. The process of developing is following. Yeast cells transformed to produce HBsAg were cultured in a fermenter, the cell debris was mechanically disrupted and the cell-free lysate was treated with procedures of salfing out, isoelectri point precipitation, gel chromatography, ion-exchange chromatography and ultracentrifuge fractionation. The purified HBsAg (YHBs) was thus obtained. Then antigen solution was treated with 0.05% formalin at 37$^{o}$C for 96 hours and was adsorbed onto aluminum hydroxide gel at appropriate protein concentration. Finally thimerosal was added as preservative and dispensed into vials for HB vaccine as the final preparation.

**Hepatitis B Immunoglobulin**

It was purchased from National Blood Center, Thai Red Cross Society.

**Immunization protocols**

Healthy infants born to HBsAg and HBeAg positive mothers were randomly assigned into 4 groups to receive the HB vaccine (Bimmugen 10 ug HBsAg/0.5 ml Kaketsuken, Japan) With or without the HBIG (National Blood Center, Thai Red Cross Society 100 IU/ml). Primary 3 doses of HB vaccine were administered by the muscular route at birth, 1 month and 2 months after birth. Group A received 10 ug HB vaccine only, group B received 10 ug HB vaccine plus 1 ml of HBIG at birth, group C received 5 ug HB vaccine only and group D received 5 ug HB vaccine plus 1 ml of HBIG at birth. Group C and D received a booster dose of 5 ug vaccine at one year of age.

**Blood sample colection**

Pregnant women blood samples were collected for screening for HBsAg and HBeAg. Blood samples from vaccinated infants were drown from the venous blood of the infants at birth before immunization, 2 months, 4 months, one year of age and 13 months of age (only in group C and D).

**Adverse reactions**

Adverse reactions to the vaccination were recorded for the following 3 days in hospital on individual symptom sheets by the physicians.

**Laboratory methods**

Screening of pregnant wormen for HBsAg and HBeAg were performed by reversed passive haemagglutination (RPHA) with RPHA reagent (Virus Research Institute, Department of Medical Sciences, Bangkok, Thailand) and anti-e cell Neo (The Green Cross Corporation, Osaka), respectively. Post vaccinated samples were tested for anti-HBs by enzyme linked immunosorgent assay (ELISA, AUSAB Abbott) and for HBsAg by RPHA. Titers of anti-HBs were expressed in milli-international units per millilitre (mIU/ml.)

**Statistical analysis**

Significant tests were done by the Chi-square method and Fried-Man-two way Anova test.

# Results

**Maternal screening**

Seven thousand one hundred and fifty-three pregnant women from Royal Navy Hospital were screened for HBsAg by RPHA method. Three hundred and forty six HBsAg positive sera (4.84%) were further tested for the presence of HBeAg by RPHA and 153 (44.22%) HBeAg positive mothers were selected for the trial.

**Anti-HBs response to vaccination in infants**

One hundred and twelve infants were born from 153 HBeAg positive mothers but only 90 infants enrolled this study. In most cases, dropout from the study was due to migration of the parents. None of the 90 infants developed hepatitis B virus infection throughout the duration of follow-up because no HBaAg was found in the infants but they developed antibody against HBV infection. The immune responses to HB vaccination in the four groups are shown in table 1. There was no statistically significant difference in geometric mean titers (GMT) ($p > 0.05$ Table 2).

The results in 15 (group A), 22 (group B), 27 (group C) and 26 (group D) neonates showed that two months after the first dose of vaccine, 60.0% (9/15), 95.5% (21.22), 81.5% (22/27) and 92.3% (24/26) seroconverted with anti-HBs geometric mena titers of 10.63, 63.10, 38.89 and 59.90 mIU/ml, respectively. However at two months after the third dose 93.3% (14/15), 100% (20/20), 95.8% (23/24) and 96.3% (26/27) seroconverted with anti-HBs GMT of 113.6 mIU/ml, 104 mIU/ml, 102.01 mIU/ml and 160.51 mIU/ml, respectively. After follow up for one year each of the four groups was 100% seroconverted 13/13, 22/22, 21/21 and 23/23 (Table 2) with anti-HBs GMT of group A 168.17 mIU/ml, group B 184.48 mIU/ml, group C 143.62 mIU/ml and group D 265.40 mIU/ml. Only for group C and D were received booster dose at one year and one month later, We found the high increasing of anti-HBs GMT of group C 2315.81 mIU/ml and group D 2577.44 mIU/ml, respectively. There were no statistically significant differences in anti-HBs response between group A and C, group C and D ($P > 0.05$) but there was significant difference in anti-HBs between group A and B at two months of age ($P < 0.05$).

**Adverse reactions**

No serious side-effect from the vaccination was observed. No case of acute hepatitis had occurred. The most common local symptom was only redness at the site on injection. The infants were healthy and had only the usual intercurrent illness.

**Table 1 The seroconversion rategafter HB vaccination alone or in combination with HBIG in high risk infants.**

| Group of vacciness | Age of vaccinees | | | |
|---|---|---|---|---|
| | 2 months | 4 months | 1 year | 13 months |
| Group A | 9/15* | 14/15 | 13/13 | - |
| | 60%** | 93.3% | 100.0% | |
| Group B | 21/22 | 20/20 | 22/22 | - |
| | 95.5%** | 100.0% | 100.0% | |
| Group C | 22/27 | 23/24 | 21/21 | 18/18 |
| | 81.5% | 95.8% | 100.0% | 100.0% |
| Group D | 24/26 | 26/27 | 23/23 | 18/18 |
| | 92.3% | 96.3% | 100.0% | 100.0% |

A : vaccine 10 ug alone,

B : vaccine 10 ug + HBIC

C : vaccine 5 ug alone

D : vaccine 5 ug + HBIG

* 9/15 = No. of infants with anti-HBs/total no. tested

** Statistically significant by Chi-square test $P < 0.05$ Gr.A VS Gr.B

**Table 2 The geomtric mean titres of Anti-HBs in various groups of high risk infants.**

| Group | Geometric mean titres of Anti-HBs mIU/ml | | | |
|---|---|---|---|---|
| | 2 months of age | 4 months of age | 1 year of age | 13 months of age |
| Group A | 10.63 | 113.6 | 168.17 | - |
| Group B | 63.10 | 104.0 | 184.48 | - |
| Group C | 38.84 | 102.01 | 143.62 | 2351.81 |
| Group D | 59.90 | 160.51 | 265.40 | 2577.44 |

No statistically significant difference by Fried-Man two way Anova test $P > 0.05$

## Discussion

In the present study, vaccination with a recombinant DNA yeast-derived vaccine was shown to be safe and immunogenic in high risk neonates for hepatitis B infection. No serious adverse reaction was observed after vaccination and all vaccinated infants who did not become chronic carriers produced high titers of anti-HBs. The booster dose of vaccine at month 12 will probably extend high immunogenicity.

Infants who responded well to the vaccination had titers over the generally accepted protective level of 10 mIU/ml at one month after the second dose.

Hayashi et al (1988) reported that 5 ug of the same vaccine used in Japanese children induced a high anti-HBs seroconversion rate and high antibody titer. When compared to the result of the other vaccine, Poovorawan et al (1989) reported that infection rate of high infections Thai infants who received a 10 ug dose of SKF recombinant vaccine within 12 hours of birth and additional dose 1, 2 and 12 months later was 3.6% whereas there was no carrier infant in our study, but high seroconversion rate and high anti-HBs titers were obtained from this study. We found that there was no significant difference between 5 ug dose and 10 ug dose However, seroconversion rate of vaccinated infants in group C was higher than those in group A and B (from Table 1). It may be in group C composed of high responder vaccinated infants. Because of the cost of the vaccine we should use 5 ug dose as the optimum dose in mass immunization of newborns.

our results were similar to Preyapan's reported (Sangaron, 1989) in using HB-vax II 2.5 and 5 ug in neonates. She found that there was no significantly difference in seroconversion rate between two groups and no carrier infants. Geometric mean titer of 5 ug dose was 281/ mIU/ml at 6 months of age and raised to 1401 mIU/ml at 8 months of age. While our geometric mean titer of 5 ug dose was 160.51 mIU/ml at 4 months of age and raised to 2577.44 mIU/ml at 13 months of age after a booster dose.

Compartive immunogenicity of vaccine 5 ug alone and in combination of HBIG was not statistically significant between group C and group D (Table 1). Due to a small sample size we could not say that vaccine 5 ug alone was more suitabel than vaccine in combination of HBIG in preventing perinatal transmission of hepatitis B virus infection. However we should continue to use vaccine in combination of HBIG for the best protection. Moreover for economic or there are problems for supply of HBIG, vaccine 5 ug alone may be accepted for the protection.

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# Comparative Immunogenicity of Recombinant DNA Hepatitis B Vaccine Alone or in Combination with Hepatitis B Immunoglobulin in Newborns of HBe Antigen Positive Mothers.

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## Abstract

During 1988-1991 a clinical trial to interrupt transmission of hepatitis B virus (HBV) from HBsAg and HBeAg positive mothers to their infants was attempted in 90 infants born to HBsAg/HBeAg positive mothers at the Royal Navy Hospital, Bangkok, Thailand. Subjects were randomized into 4 groups. Group A received 10 ug yeast-derived hepatitits B vaccine (HB vaccine) alone while group B received 10 ug HB vaccine in combination with hepatitis B immunoglobulin (HBIG) at birth. Group C received 5 ug HB vaccine alone and group D received 5 ug HB vaccine plus HBIG at birth. HB vaccine was given at birth, 1, 2 months after birth and group C and D received booster dose at one year. The antibody to HBsAg (anti-HBs) positivity rate in sera of group A, B, C and D infants at 4 months of age were 93.3%, 100%, 95.8% and 96.3%, respectively. The geometric mean titers (GMT) of anti-HBs were 113.6 mIU/ml, 104 mIU/ml. 102.01 mIU/ml and 160.51 mIU/ml in group A, B, C and D infants, respectively. At one year of age each of the four groups was 100 percent seroconverted with anti-HBs GMT 168.17, 184.48, 143.62, and 265.40 mIU/ml of group A, B, C and D respectively. There were no statistically significant among the four groups in anti-HBs GMT, No adverse reactions were observed after immunization with HB vaccine and HBIG. We conclude that the vaccine is high immunogenic and safe for use in newborns alone or in combination with HBIG. It

was found that vaccine 5 ug dose was the optimum dose and induced the same result as vaccine 10 ug dose. For economic point of view, vaccine 5 ug dose is recommended for immunization.

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Key words : Clinical trial, yeast-derived hepatitis B vaccine, Hepatitis B immunoglobulin, Immunogenicity.

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