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# EVALUATION OF THE IMMUNOGENICITY AND REACTOGENICITY OF A RECOMBINANT YEAST– DERIVED HEPATITIS B VACCINE (TEMREVAC-HB)

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## ABSTRACT

The immunogenicity and reactogenicity of a yeast derived recombinant vaccine (TEMREVAC-HB) was evaluated. A total of 91 subjects, ages 1-67 years old (46 were children, 45 were adults), were enrolled in the trial and were given TEMREVAC-HB using the 5 ug (1-19 years) and 10 ug (over 19 years) dose in a 3-dose vaccine series at 0,1 and 6 months. Sera to determine antibody titers were evaluated twice at 3 months after 1st injection (T3) and 7 months after the 1st infection (T7). A seropositive titer was defined as ≥10 mIU/ml. At T3, seroprotection was 70% and 30% respectively in children and adults. Geometric mean titers (GMT) revealed 1144.6 and 690.4 respectively. At T7, an anti-HBs titer over 10 mIU/ml was 100% and 91% respectively. GMT's of the anti-HBs antibodies were 1621.8 and 1259.2 respectively. The mean Anti-HBS antibody titer count of the pediatric group was higher by 364.7347 than that of the adult group. Only 3 out of 91 subjects experienced local and systemic reactions such as pain and vomiting. It is to be concluded in this present trial that the yeast derived recombinant vaccine, Temrevac-HB, using the 5 ug in children and 10 ug in adults in a 3 dose vaccine series at 0,1 and 6 months was proven, based on statistical analysis, to be highly immunogenic and safe.

## INTRODUCTION

Worldwide, infection with the hepatitis B virus (HBV) remains to be a major public health concern. More than 2000 million people have been infected with the virus. Approximately 300 million are chronically infected and are at high risk of serious illness, around 500,000 to 750,000 individuals die every year from cirrhosis and primary liver carcinoma, the 2 major sequelae of hepatitis B infection[1]. In the Philippines, various surveillance reports show an increasing number cases of hepatitis B. It is most interesting to note that Filipinos are infected before the age of six years but most cases manifest within the age group of 20-49 years. Approximately 12% of Filipinos are chronic carriers.[13]

Hepatitis B is preventable with a safe and effective vaccine. In fact, hepatitis B vaccine is considered the first vaccine against cancer. Since the discovery of the hepatitis B virus 35 years ago, vaccination remains to be the key preventive measure against Hepatitis B infection. A comprehensive strategy to prevent HBV infection and its sequelae must include elimination of transmission that occur at birth and during childhood as well as during adolescence and adulthood. Universal infant immunization is now recognized as the most effective proper strategy for every country for the long term control of chronic HBV infection and its sequelae.[11] According to the World Health Organization (WHO), the priorities for hepatitis B immunization strategies in order of importance include routine infant vaccination, prevention of perinatal HBV transmission and catch-up vaccination for older age groups.[13]

It was in 1993, the vaccine was incorporated in the Expanded Program of Immunization (EPI) in our country. The integration of hepatitis B vaccine in existing childhood vaccination schedule has the likelihood of long-term success[1]. In countries with high endemicity (HbsAg prevalence 8% or more), as the Philippines, WHO recommended the integration of the hepatitis B vaccine in its national immunization program. However, many low income countries in Sub-saharan Africa and the Indian subcontinent still have difficulty using the vaccine. Vaccine cost has been one of the main obstacles to its introduction in many of these countries. Logistical difficulty associated with implementing such national programs is likewise a major problem. Even in countries where Hepatitis B vaccine has already been incorporated in its national immunization program, vaccine cost remains to be a major deterrent in making the vaccine available to the general population.

At present, there are two types of hepatitis B vaccine which are commercially available . Hepatitis B vaccines are either plasma derived or synthesized using recombinant technology. However, the use of plasma-derived vaccines have decreased and more developed countries use the recombinant vaccine. The recombinant or genetically engineered vaccines are made using HBsAg synthesized in yeast (*Saccharomyces cerevisae*) or in mammalian cells into which the HBsAg gene has been inserted. Each country has a different preparation.

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The recombinant vaccines contain 5 to 40 ug of HBsAg protein per ml adsorbed to aluminum hydroxide. Although the concentration of recombinant HBsAg protein differ among vaccine products, rates of seroconversion are equivalent when given to immunocompetent infants, children, adolescents, or young adults in the doses recommended[2]. The use of a lower vaccine dose in adolescents and adults (10 ug) and in infants and children (5 ug) with the same efficacy and safety leads to a significant reduction in the cost of hepatitis B vaccination which leading to easier integration in immunization programs.

The objective of the present study is to evaluate the immunogenicity and reactogenicity of a yeast-derived recombinant vaccine (Temrevac-HB) using the 5 ug dose (age 1-19 years) and 10 ug dose (over 19 years old). Although several clinical trials have already proven the efficacy and safety of the lower vaccine dose, this study was conducted to have our own local experience in the country.

## MATERIALS AND METHODS

### Study Population

The study was conducted among residents of Metro Manila, particularly Las Pinas, Muntinlupa and Taguig . Some of the subjects are residents of Cavite and Batangas. Prior to enrollment, the conduct of the trial was explained to the adult participants and the children's parents and a written consent form was signed.

A total of 91 subjects with ages ranging from 1-67 years were enrolled in the study. Forty six subjects belonged to the pediatric age group (age 1-19) and 45 participants belonged to the adult population (over 19 years old). To be included in the study, the subjects must be HBsAg, Anti-HBsAg and Anti-HBc negative as demonstrated by ELISA testing. Individuals suffering from acute to severe illness, with known immunocompromising disease and with known hypersensitivity to yeast or any component of the vaccine were excluded from the trial.

### Vaccine

Temrevac-HB is a recombinant Hepatitis B vaccine manufactured by Beijing Tiantan Biologicals Products Co. Ltd. Temrevac-HB with Certificate of Product registration no. YZ-014301 and BR – 528 . The product was produced in accordance to the technology used by Merck Research Laboratories also known as Merck Sharp and Dohme. The product is currently sold in China.

Children (age 1-19 years old) were given the Temrevac-HB 5 ug /0.5 ml dose and adults (over 19 years old) were given the Temrevac-HB 10 ug/1.0ml dose using the three-dose schedule of 0, 1, and 6 months. The vaccine was administered through intramuscular injection by registered nurses of the University of Perpetual Help Medical Center. Injections were given in the deltoid muscle for children more than 2 years old and adults and in the anterolateral aspect of the thigh in infants.

### Serology

Serological analysis was conducted on blood samples drawn on two occasions. Sera was collected 3 months (T3) and 7 months (T7) after the first injection. Anti-HBs antibodies were determined using an ELISA diagnostic kit and conducted by an independent laboratory. The assay cut-off was 10 mIU/ml. A seropositive titer was defined as ≥10 mIU/ml.

### Reactions

Local and systemic reactions were observed within 72 hours after injection A monitoring form was given to the participants and children's parents taking note of local reactions such as swelling, pain, tenderness and redness and systemic reactions such as fever ($T^o \geq 37.5^oC$/axilla), headache, diarrhea, rashes nausea and vomiting.

### Statistical Analysis

Geometric mean titers and seroconversion rates were calculated. The SPSS program was used to generate the other statistical results. Chi square was manually calculated to determine the independence between T3, T7 and age.

## RESULTS

A total of 91 subjects were enrolled in the trial 46 were children ( 0-19 years) and 45 were adults (over 19 years). Males constitute 33 % and females 67 %. (Table 1)

**Table 1. Number of subjects based on age and gender**

| | Male | Female | Total |
|---|---|---|---|
| Children ( 0-19 years) | 16 | 30 | 46 |
| Adults ( over 19 years) | 14 | 31 | 45 |
| **Total** | **30** | **61** | **91** |

## Immunogenicity

Using a one sample T-test there is sufficient evidence to support that the mean antibody titer produced in children for **T3** and **T7** is at least 10 mIU/ml (Tables 2a and 2b)

**Table 2a and 2b. Geometric Mean Titer and confidence interval at T3 and T7 for children**

| Table 2a | Test Value = 10 | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 99% Confidence Interval of the Difference | |
| | t | df | Sig. 2-tailed | Mean difference | Lower | Upper |
| Children's antibody titer 3 months after 1st injection | 6.455 | 45 | .000 | 1134.6285 | 661.84404 | 1607.413 |

| Table 2b | Test Value = 10 | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 99% Confidence Interval of the Difference | |
| | t | df | Sig. 2-tailed | Mean difference | Lower | Upper |
| Children's antibody titer 7 months after 1st injection | 20.415 | 45 | .000 | 1611.8239 | 1399.475 | 1824.173 |

Likewise, using the One-Sample T-test, there is sufficient evidence to support that the mean antibody titer produced for **T3**and **T7** is at least 10 mIU/ml for adult subjects (Tables 3a and 3b)

**Table 3a and 3b . Geometric Mean Titer and confidence interval at T3 and T7 for adults**

| Table 3a | Test Value = 10 | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 99% Confidence Interval of the Difference | |
| | t | df | Sig. 2-tailed | Mean difference | Lower | Upper |
| Adult's antibody titer 3 months after 1st injection | 4.660 | 44 | .000 | 1134.6285 | 680.35702 | 1073.420 |

**Table 3a and 3b . Geometric Mean Titer and confidence interval at T3 and T7 for adults**

| Table 3b | Test Value = 10 | | | | | |
|---|---|---|---|---|---|---|
| | | | | | 99% Confidence Interval of the Difference | |
| | t | df | Sig. 2-tailed | Mean difference | Lower | Upper |
| Adult's antibody titer 7 months after 1st injection | 13.077 | 44 | .000 | 1247.1559 | 990.38506 | 1503.927 |

At T3 (3 months after the first injection), seroprotection was 70% and 30% respectively in children and adults. Geometric mean titers (GMT) revealed 1144.6 and 690.4 respectively. (Table 4)

At T7 (7 months after 1st injection), an anti-HBs titer over 10 mIU/ml was 100% and 91% respectively. GMT's of the anti-HBs antibodies were 1621.8 and 1259.2 respectively. (Table 4). The mean Anti-HBS antibody titer count of the pediatric group was higher by 364.7347 than that of the adult group.

Titers of Anti-HBS antibodies show that 58 out of 91 or 64% of the subjects have already become reactive to TEMREVAC-HB Vaccine 3 months after the 1st injection (T3), and 87 out of 91 or 96% for T7 (7 months after the 1st injection). Four subjects had antibody titers less than 10mIU/ml. All seronegative cases belong to the adult population.

**Table 4. Percentage of Seropositive Subjects and anti-HBs Geometric Mean Titers (GMT)**

| Age Group | Antibody Titer (T3) | | Antibody Titer (T7) | |
|---|---|---|---|---|
| | children | adult | children | adult |
| GMT | 1144.629 | 690.357 | 1621.824 | 1257.156 |
| Standard deviation | 1192.221 | 979.373 | 535.481 | 639.782 |
| # seropositive | 32 | 26 | 46 | 41 |
| # seronegative | 14 | 19 | 0 | 4 |
| **Total** | **46** | **45** | **46** | **45** |
| Seropositive rate | 70% | 58% | 100% | 91% |
| Seronegative rate | 30% | 42% | 0% | 9% |

A test for correlation between the antibody titer for T7 and age using Pearson's , Kendall's tau and Spearman's rho tests concurred a slightly inverse relationship between age and antibody titers at level alpha

0.01 and 0.05 (Tables 5 and 6), meaning upon use of TEMREVAC-HB Vaccine, as the age of the subjects increase, the antibody titer count produced are likely to be lesser. Another interpretation can be that the lower the age of the subjects who use the vaccine, the higher the antibody titer count that they were able to generate.

**Table 5. Correlation between age and antibody 7 months after injection using Pearson's test**

| | | Age of Subjects | Antibody Titer 7 months After 1st Injection |
|---|---|---|---|
| Age of Subjects | Pearson Correlation | 1.000 | -.288** |
| | Sig. (2-tailed) | - | .006 |
| | N | 91 | 91 |
| Antibody Titer 7months After 1st Injection | Pearson Correlation | -.288** | 1.000 |
| | Sig. (2 tailed) | .006 | - |
| | N | 91 | 91 |

**Table 6. . Correlation between age and antibody 7 months after injection using Kendall's tau and Spearman's rho**

| Correlations | | | | |
|---|---|---|---|---|
| | | | Age of Subjects | Antibody Titer 7 months After 1st Injection |
| Kendall's tau_b | Age of Subjects | Correlation Coefficient | 1.000 | -.160* |
| | | Sig. (2-tailed) | - | .026 |
| | | N | 91 | 91 |
| | Antibody Titer 7 months After 1st Injection | Correlation Coefficient | -.160* | 1.000 |
| | | Sig. (2-tailed) | .026 | - |
| | | N | 91 | 91 |
| Spearman's rho | Age of Subjects | Correlation Coefficient | 1.000 | -.253* |
| | | Sig. (2-tailed) | - | .016 |
| | | N | 91 | 91 |
| | Antibody Titer 7 months After 1st Injection | Correlation Coefficient | -.253* | 1.000 |
| | | Sig. (2-tailed) | .016 | - |
| | | N | 91 | 91 |

*Correlation is significant at the .05 level (2-tailed).

A chi-square ($\chi^2$) test for independence between **T3** and **gender** of subjects and **T7** and **gender** was also performed. Results showed no significant correlation; meaning the antibody titer count (**T3** and **T7**) achieved was independent of gender. (Table 7)

**Table 7. Seropositive rate based on gender at T3 and T7**

| | REACTION | |
|---|---|---|
| **GENDER** | **Seropositive (T3)** | **Seropositive(T7)** |
| **Male** | **17** | **26** |
| **Female** | **41** | **61** |
| **Total** | **58** | **87** |

**Reactogenicity:**

Out of 91, only 2 subjects reported local reactions such as pain (9 year old male and 26 year old female) on the day of vaccination; and only one subject (7 year old male) reported pain and vomiting. No other local and systemic reactions were observed from the other subjects after vaccination.

## Discussion

Yeast-derived recombinant hepatitis B vaccine have largely replaced plasma-derived vaccines in developed countries and have now been given to millions of individuals worldwide. However, the high cost of the vaccine remains to be a major obstacle to its use and therefore a significant number of the population remains to be at risk. Vaccine cost, apart from other logistic difficulties, has to be addressed especially if a successful integration of the hepatitis B vaccine into national immunization programs is sought for. One of the strategies that may be used to counteract cost is through administration of a lower vaccine dose which is highly immunogenic and safe.

Different studies on the immunogenicity of hepatitis B vaccine result to a wide range of seroprotection and Anti-HBs geometric mean titers (GMTs)[3,4,6,7,8,10] due to different vaccine characteristics and formulation, vaccine schedules, timing of blood sampling and sensitivity of laboratory methods. In the present study, with the use of a lower vaccine dose, immunogenicity was demonstrated with seroconversion at 7 months after the first injection (T7) in 100% of children and 91% of adults. The GMTs of Anti-HBs observed in the vaccines were 1144.6 and 690.4 at T3 respectively and 1621.8 and 1259.2 respectively at T7. Similar studies comparing the lower vaccine dose of 5 ug for children and 10 ug for adults with the more widely used dose of 10ug (children) and 20 ug (adults) resulted in seroconversion rates from 80-100%[3,4,6,7,8,10]. At T3 (3 months after the first injection), seroprotection was 70% and 30% respectively in children and adults. This data just shows that protective levels of antibody in a greater proportion of the subjects is only achieved after completion of the 3-dose vaccine series at 6 months.

Age associated changes in immune function may contribute to decreased vaccine efficacy in older individuals, although researches in this area have yielded contradictory findings. In the present study, correlation studies concurred a slightly inverse relationship between age and antibody titers. It was also noted that all four of the non-responders belong to the adult population and

the geometric mean antibody titers were higher in children compared to that of the adults. A meta-analysis of 24 published trials evaluated the association of age and response to hepatitis B vaccination and pooling of the study results suggested a significantly increased risk of nonresponse to hepatitis B among older individuals[5]. This may have important implications especially to health care workers, travelers and high risk adults who will be vaccinated for the first time. In an article by Yu, et al, they have found predictors of non-response to hepatitis B vaccine to include the following: increasing age , male gender, obesity, tobacco smoking and immunocompromising chronic disease[12]. Some of these factors mentioned however, such as obesity and tobacco smoking were not assessed in our study population and subjects were limited to immunocompetent individuals.

In this study, the antibody titer count (at **T3** and **T7)** achieved was independent of gender. Of the 4 non-responders 2 were female and the other 2 were male. This result is somehow in conflict with what is usually reported in literature that the male gender is one of the risk factors for non-response in hepatitis B vaccination. As was mentioned earlier other factors which could have affected the results were not investigated.

Adverse effects, both local and systemic, related to vaccination were very minimal. Only 3 out of the 91 subjects reported reactions such as pain (in all 3) and vomiting (in only one). There were no reported serious adverse effects that warranted subject discontinuation from the study. In numerous clinical trials, only minimal adverse effects were also reported, most common as was seen in our study, pain on injection site[7].

## Conclusion and Recommendations

It is to be concluded in this present trial that the yeast derived recombinant vaccine, Temrevac-HB, using the 5 ug in children and 10 ug in adults in a 3 dose vaccine series at 0,1 and 6 months was proven, based on statistical analysis, to be highly immunogenic and safe. These findings may have significant implications in terms of vaccine cost savings.

The following recommendations are put forward:

1. conduct of the trial to a larger number of subjects, both adults and children
2. follow-up study to determine the duration of protection and the potential need for booster doses
3. trial involving neonates (term and preterm, normal and high risk) to be able to assess its potential benefit in interrupting perinatal transmission of hepatitis B.

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**Unpublished articles:**


1. Immune Effect of Recombinant Yeast Hepatitis Vaccine
2. Safety an dimmunogenicity of recombinant yeast-derived hepatitis B vaccine in infants immunization
3. A clinical Trial for Safety and Efficacy of domestic Recombinant Hepatitis B Vaccine
4. Evaluation on safety and immunogenicity of recombinant yeast-derived hepatitis B vaccine