THE IMMUNOGENICITY OF LOW DOSE INTRADERMAL RECOMBINANT HEPATITIS B VACCINATION, COMPARED TO STANDARD DOSE

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ABSTRACT

Hepatitis B virus (HBV) infection is still a world wide health problem. The high cost of HB vaccine is still a serious economical constraint to the prevention of the disease. The effectiveness of the low dose intradermal (LDI) methods of HB vaccination is still in debate.

This study was intended to assess the proportion of Anti-HBs seroconversion produced by low dose intradermal (LDI) recombinant Hepatitis B (HB) vaccination, compared to standard dose intramuscular (SD).

The study used a randomized controlled trial. Healthy personnel of Dr.Karadi General Hospital and senior medical student of Diponegoro University in Semarang, who seronegative to HB virus and fulfilled the eligibility criteria. Total 288 study subjects were randomized divided into two groups: LDI (experimental) group and SD (control) group. They received 3 times injection of recombinant HB vaccine (Bimmugen R) at 0, 1 and 6 months. In LDI group they received 0.1 ml (2 ug HBsAg) of vaccine injected intradermally and in SD group they received 0.5 ml (10 ug HBsAg) of vaccine injected intramuscularly. The main outcome was the Anti-HBs seroconversion (Anti-HBs > 10 mIU/mL), measured at 1 month after the last injection of HB vaccine.

It was revealed that 249 (96.5%) subjects were completely followed study protocol, consisted of 125 in LDI group and 124 in SD group. The proportion of Anti-HBs seroconversion in LDI group was 96% (95% CI: 93-99%) and in SD group was 98.4% (95% CI: 96-100%). Comparison of the proportion of Anti-HBs seroconversion between two groups were not significantly different (p = 0.23). It was also concluded that low dose intradermal recombinant HB vaccination produced the Anti-HBs seroconversion equal or comparable to those produced by standard dose intramuscular.

Suggestion: Low dose intradermal recombinant HB vaccination, which is relatively low cost and easy to conduct, may be feasible and useful as an alternative method for mass HB immunization program to adult people. This method may increase the coverage of immunization and may provide community herd immunity to Hepatitis B virus.

Keywords: low dose intradermal — recombinant Hepatitis B vaccination — randomized controlled trial — comparison — Anti-HBs seroconversion.

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INTRODUCTION

Hepatitis B virus (HBV) infection is still a worldwide health problem. Indonesia is an intermediate or high-endemicity of HBV infection. Serologic studies to healthy people showed that the prevalence of HBsAg were varied widely, ranging from 5% to 10% (Soewignyo, 1996). HBV is an environmental hazard among people and an occupational hazard among health care workers in certain settings, that is why they should be vaccinated to prevent them from this infection (Hu et al., 1991).

The high cost of HB vaccine is still a serious economic constraint to the extensive vaccination needed in many countries. The cost reduction strategy of HB vaccination by using low dose (2 ug HBsAg) intradermal (LDI) method of HB vaccination had been conducted in several countries such as by Miller et al. (1983), Redfield et al. (1985), Lancaster et al. (1985), Bryan et al. (1990), Asanteerawat et al. (1991), Manyike et al. (1992), Thompson et al. (1992), and in Indonesia by Prasetya et al. (1986), Hadi et al. (1987), and Purba et al. (1989). These study results, mostly using uncontrolled trials, showed that LDI produced Anti-HBs seroconversion (Anti-HBs > 10 mIU/ml) 63% - 97.3%. Previously, the result of standard dose (recommends by factory) injected intramuscularly of HB vaccination (Crosnier et al. 1981; Szmuness et al., 1980; Desmyter et al., 1983) showed the proportion of Anti-HBs seroconversion were 60% - 100%. Some researchers who conducted LDI method and got good results, concluded that their results were equal to or comparable to the standard dose intramuscular (SD). However, due to improper research methodology used, some experts were still doubtful about these results. Randomized controlled trials (RCT) to compare LDI and SD HB vaccinations with adequate sample size are still needed to answer this controversial issue.

This comparative study using RCT design was conducted to assess the proportion of Anti-HBs seroconversion produced by LDI recombinant HB vaccination, compared to SD. The hypothesis of this study was: there is no difference in the proportion of Anti-HBs seroconversion between LDI and SD recombinant HB vaccination.

MATERIAL AND METHOD

Study subject

Not less than 669 volunteers from Dr. Kariadi General Hospital and senior medical students of Diponegoro University in Semarang,
Indonesia were screened for HBV seromarkers to recruit candidates of study subjects. Minimal sample size calculation was fixed by using the formula of sample size requirement for hypothesis testing of 2 populations proportion (Lemeshow et al., 1990), with $\alpha = 0.05$ (one-sided), $\alpha = 0.20$, and drop-outs 10%. The minimal sample size for this study was 246. A total number of 258 healthy subjects who were susceptible to HBV (negative HbsAg and negative Anti-HBs) and fulfilled the eligibility criteria, were enrolled in this study.

**Study protocol**

After signing the informed consent form and randomized by random permuted block of twenty, they were divided into two groups of intervention, consisted of 130 subjects in LDH group as an experimental group and 128 subjects in SD group as control group. They received 3 times injection of recombinant HB vaccine (Binnmugen R/) at 0.1 and 6 months period. The same 1 mL tuberculin/insulin syringe and 26G (0.45x12mm) needle were used for vaccine injection, but with difference dose and route of HB vaccine. In LDH group they received 0.1 mL (2 ug HbsAg) of vaccine injected intradermally and in SD group they received 0.5 mL (20 ug HbsAg) of vaccine injected intramuscularly. Anti-HBs seromarker was measured at 1-month after the last injection of vaccine, and tested by commercial enzyme-linked immunoassay (Boehringer Mannheim, Germany). The immunogenicity of HB vaccination was defined as the Anti-HBs seroconversion expressed by the conversion of Anti-HBs from negative before vaccination to positive after vaccination with Anti-HBs titre equal to or more than 10 mIU/mL.

**Statistical analysis**

The proportion of Anti-HBs seroconversion comparison between two groups of study was tested by using Chi-square test. Statistical significance was referred to $p < 0.05$ (one-sided), confidence limit (CI) 95%, and power 80%. SPSS/PC program was used for statistical analysis.

**RESULTS AND DISCUSSION**

Among 258 study subjects, 9 subjects (3.5%) were dropped-out and 249 subjects (96.5%) completely followed all study protocol and could be analyzed. Baseline data comparison between two groups of study were not significantly difference ($p > 0.05$) according to the number of subjects, the mean of age, and the mean of body mass index (BMI), except for sex distribution. The proportion of male and female subjects between two groups were significantly difference ($p = 0.02$) (Table 1).
Table 1. Baseline data comparison between LDI group and SD group (N = 249)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>LDI Group</th>
<th>SD Group</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects</td>
<td>125 (50.2%)</td>
<td>124 (49.8%)</td>
<td>ns, p=0.51</td>
</tr>
<tr>
<td>Male / Female</td>
<td>36 / 89</td>
<td>54 / 70</td>
<td>s, p=0.02</td>
</tr>
<tr>
<td>Age (Years)*</td>
<td>30.02 (8.13)</td>
<td>30.45 (7.86)</td>
<td>ns, p=0.37</td>
</tr>
<tr>
<td>Body Mass Index*</td>
<td>21.87 (3.13)</td>
<td>22.11 (3.16)</td>
<td>ns, p=0.27</td>
</tr>
</tbody>
</table>

Note: * = Mean (Standard Deviation); s = significant; ns = not significant

In this study, the proportion of Anti-HBs seroconversion in LDI group was 96% (95% CI: 93-99%), whereas in SD was 98.4% (95% CI: 96-100%), respectively. Comparison of the proportion of Anti-HBs seroconversion between two groups of study did not show a significant difference (p = 0.23) (Table 2).

Table 2. Comparison of the proportion of Anti-HBs seroconversion between LDI group and SD group (N = 249)

<table>
<thead>
<tr>
<th>Anti-HBs seroconversion</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDI Group</td>
<td>120 (96%)</td>
<td>5 (4%)</td>
<td>125 (100%)</td>
</tr>
<tr>
<td>* (95% CI: 93-99%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD Group</td>
<td>122 (98.4%)</td>
<td>2 (1.6%)</td>
<td>124 (100%)</td>
</tr>
</tbody>
</table>

p = 0.23

Subgroup analysis comparison of Anti-HBs seroconversion between male and female subjects between two groups were not significantly difference (male subjects p = 0.17 and female subjects p = 0.59) (Table 3).

Table 3. Comparison of Anti-HBs seroconversion among male and female subjects between two groups of study (N=249)

<table>
<thead>
<tr>
<th>Sex Category</th>
<th>Anti-HBs Seroconversion</th>
<th>Positive</th>
<th>Negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>LDI Group</td>
<td>33 (91.7%)</td>
<td>3 (8.3%)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>SD Group</td>
<td>53 (98.1%)</td>
<td>1 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>LDI Group</td>
<td>87 (97.8%)</td>
<td>2 (2.2%)</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>SD Group</td>
<td>69 (98.6%)</td>
<td>1 (1.4%)</td>
<td></td>
</tr>
</tbody>
</table>
Weber et al. (1995) and Horowitz et al. (1998) found in their retrospective studies that old-age subject (more than 39 years-old, and high BMI subject (BMI > 25, or overweight and obesity) were associated with poor or non-response (Anti-HBs < 10 mIU/mL or negative Anti-HBs seroconversion) to HB vaccine. Those conditions did not occur in this study, the proportion of Anti-HBs seroconversion among old subjects was 94.1% in LDI group and 100% in SD group; and among high BMI subjects was 92.9% in LDI group and 95.5% in SD group, respectively.

This result supported the hypothesis of study that there is no difference in the proportion of Anti-HBs seroconversion between LDI and SD recombinant Hepatitis B vaccination. This study had been conducted by RCT with adequate sample size, and the result may be able to convince someone who is still doubtful about the efficacy of LDI recombinant Hepatitis B vaccination in producing Anti-HBs seroconversion.

Previous results of LDI recombinant Hepatitis B vaccination showed a wide range of Anti-HBs seroconversion from 63% to 95% (Morris et al., 1989; Parish et al., 1991). The wide range of Anti-HBs seroconversion might be due to the difference in study population, the different type, trade mark and dose of LDI recombinant HB vaccine, and the technique of intradermal injection of HB vaccine used. A proper technique of intradermal injection or deposition of antigen (HB vaccine) is very important to achieve a good immune response to HBV on HB vaccination (Wahl and Hermansson, 1987). By training and using 1 ml insulin syringe and 26G (0.45x12 mm) needle, it was easy to conduct intradermal injection of vaccine.

In general, LDI method produced Anti-HBs titers lower than those produced by SD. It is reasonable because a smaller dose of HB vaccine was used in LDI than those used in SD. In this study, Anti-HBs titers of study subjects were categorized into such level-grouping, modified from Jlg et al. (1994).

Table 4. The level-grouping of Anti-HBs titers among LDI group and SD group (N = 29).  

<table>
<thead>
<tr>
<th>Anti-HBs titer</th>
<th>LDI Group</th>
<th>SD Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0 - 9</td>
<td>5 (4%)</td>
<td>2 (1.6%)</td>
<td>7 (2.8%)</td>
</tr>
<tr>
<td>2. 10 - 99</td>
<td>35 (28%)</td>
<td>13 (10.5%)</td>
<td>48 (19.3%)</td>
</tr>
<tr>
<td>3. 100 - 999</td>
<td>76 (60.8%)</td>
<td>47 (37.9%)</td>
<td>123 (49.4%)</td>
</tr>
<tr>
<td>4. 1000 - 9999</td>
<td>9 (7.2%)</td>
<td>43 (34.7%)</td>
<td>52 (20.9%)</td>
</tr>
<tr>
<td>5. ≥ 10,000</td>
<td></td>
<td>19 (15.3%)</td>
<td>19 (7.6%)</td>
</tr>
</tbody>
</table>
Although Anti-HBs titers were low, by booster injection given to the subject, or by anamnestic reaction when they were in contact or infected by HBV, their immune responses or their Anti-HBs titers that they already have, will increase rapidly. This phenomenon especially occur to people who live in endemic areas of HBV (Hollinger, 1980).

In this study, LDI method used 0.1 mL (2 ug HBsAg) of recombinant HB vaccine per injection. Theoretically, it is equal to one-fifth of the standard dose of 0.5 mL (16 ug HBsAg) per vial of Bimmugen R/ HB vaccine, but in practice it is only equal to one-fourth of that vaccine. This method provided 75% reduction of HB vaccine cost in one basic course of HB vaccination.

LDI method of recombinant HB vaccination may save the cost of HB vaccine or is relatively low cost and easy to conduct. LDI method may be feasible and useful in mass HB immunization program for adult people, especially in developing countries. Mass HB immunization program using LDI method may increase the coverage of immunization and may provide the community herd immunity to HBV for the people, especially in developing countries.

CONCLUSION AND SUGGESTION

Conclusion

Low dose intradermal recombinant Hepatitis B vaccination produced an equal proportion of Anti-HBs seroconversion as that produced by standard dose intramuscular.

Suggestion

A proper technique of intradermal vaccine injection is very important to achieve a good immune response to HBV in conducting low dose intradermal (LDI) Hepatitis B vaccination. By training, using appropriate syringe and needle, it is easy to perform LDI Hepatitis B vaccination to adult people.

LDI recombinant Hepatitis B vaccination, which is relatively low cost and easy to conduct, may be feasible and useful as an alternative method for mass Hepatitis B immunization program to adult people, especially in developing countries. Mass Hepatitis B vaccination program with LDI recombinant HB vaccine may increase the coverage of immunization and may provide community herd immunity to Hepatitis B virus.
REFERENCES


