Immunoprophylaxis to limit a hepatitis B epidemic among women undergoing in vitro fertilization

Pia M. Grosheide, Helena C. van Osand, Solko W. Schalm*, Rudolf A. Heijtmak*

Women (175) who participated in an in vitro fertilization (IVF) programme were possibly exposed to hepatitis B virus. Later it became evident that 79 women had a hepatitis B infection, 49 were exposed but not infected and 47 were not exposed. Hepatitis B immunoglobulin (HB Ig) and recombinant hepatitis B vaccine (HBvaxDNA) were offered to all women and partners except for those with established hepatitis B. Women were given an 'intensive' schedule of HB Ig (0, 1 months) and vaccine (0, 1, 2, 6 months). Spouses received HB Ig (0, 1 months) and vaccine (0, 1, 6 months). Blood samples were taken at the time of diagnosis of the HBV epidemic and at regular intervals. Hospital personnel immunized according to the regular schedule (0, 1 and 6 months) with HBvaxDNA but without HB Ig served as controls. During the observation period of 7 months anti-HBc seroconversion was not observed. At month 7 the seroconversion rate for males was 89%, significantly lower than that for females (100%). Intermediate rates were found for the control groups (94%). Significant differences in geometric mean titre between IVF-treated patients and controls were, however, observed at month 7 (551 mIU ml⁻¹ for female patients versus 1582 mIU ml⁻¹ for their controls and 171 mIU ml⁻¹ for males versus 899 mIU ml⁻¹ for their controls). Possible explanations for the low reactivity to HBsAg vaccine are discussed.

Keywords: Hepatitis B postexposure prophylaxis passive active immunization

INTRODUCTION

In early March 1988 the University Hospital Dijkzigt was confronted with a hepatitis B epidemic in women who had undergone in vitro fertilization. All women (n=175) who had undergone in vitro fertilization (IVF) since November 1987 but were without signs of hepatitis B virus (HBV) infection at first screening, as well as their sexual partners, were offered passive-active immunization. The rationale for starting passive-active immunization 6–18 weeks after HBV-exposure was to modify infection in females and to prevent infection in males. All women followed an 'intensive' immunization scheme consisting of hepatitis B immunoglobulin (HB Ig) on day 1 and 1 month later and recombinant vaccine on day 1 and 1, 2 and 6 months later. Male partners who may have been exposed to HBV for several weeks also received HB Ig on day 1 and 1 month later and recombinant vaccine on day 1 and 1 and 6 months later.

In this study the levels of HBV protective antibodies (anti-HBs ≥ 10 mIU ml⁻¹) induced by passive active immunization were examined. An explanation was sought for the relatively poor immune response in the IVF population.

MATERIALS AND METHODS

Patients and controls

From 2 November 1987 until 21 January 1988, 175 women undergoing IVF might have been exposed to hepatitis B virus. Tissue culture medium supplemented with 7.5% heat-inactivated (30 min 56°C) human serum, which was used for pre-embryo cultures or other procedures during in vitro fertilization, seemed to be the most probable source of infection. The human serum was regularly pooled from blood samples of 15–20 pregnant women. All donors were individually screened for hepatitis B virus. Due to a breakdown in communication between the outpatient clinic of the Department of Obstetrics and the IVF laboratory, the results of the routine screening of sera from pregnant women for HBsAg temporarily did not reach the IVF laboratory and an HBV-positive serum was apparently included in the pooled serum. On 2 March 1988 the epidemic was discovered by reports on three cases of jaundice. All women at risk and their partners were immediately requested to visit the University Hospital Dijkzigt for medical examination. Blood samples were obtained from 174 potentially exposed women and 167 sexual partners and preventive measures were advised. These measures...
included hygiene, use of condoms during sexual intercourse and passive active immunization.

Analysis of the incidence of HBV infection in 7 day cohorts of in vitro fertilization treatment finally revealed that the period of infectious exposure only extended from 2 November to 13 December 1987 (period 1), this period coincided with the use of one of the two batches of pooled serum used in tissue culture. None of the women treated from 14 December 1987 to 21 January 1988 (period 2) developed signs of a hepatitis B infection.

Of the total of 175 women, 128 were exposed to the infectious batch of pooled serum, the remaining 47 women were not exposed to infectious culture medium. Of the 128 women exposed in period 1, 30 had clinical signs of HBV or were HBsAg-positive and pregnant at first presentation. One woman did not deliver serum and another woman was protected by previous vaccination. Therefore immunization was started in 96 women. Due to the presence of markers of hepatitis B in the pre-IVF serum (9 cases) or in serum taken at first presentation (49 cases), immunization was stopped in 58 of the 96 women (Table 1). The remaining 38 women continued immunization and may be characterized as exposed but not infected (group 1). A second group of vaccinees (group 2) is composed of women who received IVF treatment in period 2, the information that the culture medium used in period 2 was not infectious became available about 2 months after immunization had started.

Immunization was completed in the large majority of women. The other immunization groups comprised the partners. The spouses of 79 HBV-infected women by IVF treatment were labelled as group 3. The spouses of 49 non-infected women from period 1 together with the spouses of 47 women treated in period 2 comprised group 4. Further details of the four immunization groups are given in Tables 1 and 2. The control groups, hospital personnel, who served as controls, received HBVaxDNA (10 μg) from the same lot according to the regular schedule on day 1 and 6 months later without HBIG (Table 2). HBIG was administered by injection into the buttock, HB-vaccine was injected into the deltoid muscle.

Blood sampling and laboratory methods

Pre-immunization serum samples were obtained in March 1988. For females follow-up blood samples were drawn 1, 2, 6 and 7 months after the first vaccine injection. For males, blood samples were taken after 1, 4, 6 and 7 months. All pre-immunization samples from groups 1–4 as well as follow-up samples were tested for HBsAg, anti-HBc and anti-HBs. Blood samples from controls were tested for anti-HBc and anti-HBs after 7 months.

Blood samples were tested for HBsAg and anti-HBc by enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA). Anti-HBs was tested by radio immunoassay (Ausab, Abbott Laboratories) and quantified using the WHO standard preparation. A protective anti-HBs level was defined as anti-HBs concentration ≥10 mIU ml⁻¹. In the absence of other HBV markers, statistical differences in protective anti-HBs levels were assessed using the Mann-Whitney U test. The two groups with anti-HBs levels below 20 mIU ml⁻¹ at month 4 received an additional HBIG injection.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of the vaccination groups after possible exposure to hepatitis B</th>
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<tbody>
<tr>
<td>Group</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Total</td>
<td>128 (27)</td>
</tr>
<tr>
<td>Excluded at presentation*</td>
<td>32 (13)</td>
</tr>
<tr>
<td>Vaccination started</td>
<td>96 (14)</td>
</tr>
<tr>
<td>Excluded after screening*</td>
<td>58 (8)</td>
</tr>
<tr>
<td>Vaccination continued</td>
<td>38 (6)</td>
</tr>
<tr>
<td>Vaccination complete</td>
<td>29 (6)</td>
</tr>
<tr>
<td>Blood sample month 7</td>
<td>24 (3)</td>
</tr>
<tr>
<td>Evaluated month 7&quot;</td>
<td>22 (3)</td>
</tr>
</tbody>
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*Figures in parentheses indicate number of women pregnant

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics of IVF treated females, partners and controls completing hepatitis B vaccination</th>
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<tbody>
<tr>
<td>Group</td>
<td>Mean age (range)</td>
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<tr>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>1</td>
<td>Women exposed 22</td>
</tr>
<tr>
<td>2</td>
<td>Women not exposed 28</td>
</tr>
<tr>
<td>3</td>
<td>Partners 43</td>
</tr>
<tr>
<td>4</td>
<td>Partners no HBV infection 52</td>
</tr>
<tr>
<td>5</td>
<td>Female controls 84</td>
</tr>
<tr>
<td>6</td>
<td>Male controls 53</td>
</tr>
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*Sexual partner exposed but not infected (n = 20) and sexual partner not exposed (n = 32)
calculated with 95% confidence intervals for the difference between two proportions. The geometric mean titres (GMTs) for all vaccinees in various groups were compared, after logarithmic transformation by the Student’s t-test.

RESULTS

Of the 230 individuals who continued immunization after initial screening (79 females, 151 males), a total of 180 vaccinees (59 females, 121 males) completed the immunization programme. Postimmunization blood samples were obtained in 148 cases (53 females, 95 males) at month 7. Three women (2 in group 1 and 1 in group 2) were excluded from evaluation because they showed more than 10 mIU ml⁻¹ anti-HBs on day 1, although anti-HBc tests were negative.

Of the 38 females exposed to HBV (group 1), three did not show up for further treatment after month 1. At that time, they were all negative for both HBsAg and anti-HBc. Two females did not return for follow-up at month 6 but were HBsAg-negative and anti-HBs-positive at month 4; another nine women who were HBsAg-negative at month 6 and anti-HBs >10 mIU ml⁻¹ did not appear for follow-up at month 7. Of the 37 women who delivered healthy living children, none received one dose of HBlg and HBVaxDNA during pregnancy and 14 received full immunization during pregnancy. No adverse reactions to HBlg or vaccine were reported during the observation period of 7 months for vaccinees with or without HBV markers at the time of first presentation, except for one pregnant vaccinee who had a spontaneous abortion two days after initial immunization.

The rate of development of protective antibody levels (≥10 mIU ml⁻¹) is shown in Table 3. One month after the first dose of HBlg and vaccine, protective antibodies were found in 99% (203/205) of the vaccinees. Anti-HBs levels were inadequate in two cases (one female in group 2 with 8 mIU ml⁻¹ and one male in group 3 with no detectable anti-HBs). At month 2, protective antibody levels were demonstrated in all females tested. At month 4, 23% of the men of group 3 and 20% of those of group 4 were unprotected (anti-HBs <10 mIU ml⁻¹). At month 6, protective anti-HBs levels were measured significantly less often in group 4 males (48%, 95%CI 36–60%) compared with both groups 1 and 2 (87%, 95%CI 75–99%) and group 3 (81%, 95%CI 71–91%). For group 3, the higher rate of 81% was probably due to the extra dose of HBlg given at month 4 to 25 spouses with anti-HBs titres <20 mIU ml⁻¹. Protective anti-HBs levels after completion of vaccination at month 7 were found in all women of groups 1 and 2 (100%) whereas the percentage of males in groups 3 and 4 together (n=95) with protective antibodies at month 7 was 89% (95%CI 83–95%).

Table 4 shows the geometric mean titres of anti-HBs for the various groups. Antibody levels of ≥35 mIU ml⁻¹ at month 1 probably reflect the first dose of HBlg in groups 1–4. Titres rose to about 45 mIU ml⁻¹ at month 2 for women receiving two doses of HBlg and vaccine. At month 4, after two doses of HBlg and vaccine, the GMTs for groups 3 and 4 were 16 and 19 mIU ml⁻¹ anti-HBs, respectively. A significant increase in anti-HBs was not observed until after the booster dose was given. At month 7, the geometric mean titres of anti-HBs were significantly lower for all IVF groups compared with their control groups (group 1 and 2 versus 5, p<0.05, group 3 and 4 versus 6, p<0.001). Since selection of vaccinees due to loss to follow-up may have occurred, the GMT levels of all vaccinees per group were compared with the GMTs of those vaccinees who completed the full immunization scheme for each IVF group. No differences in GMTs could be shown at any moment.

At month 7 an effect of sex on the immune response to hepatitis B vaccine was indicated by a difference between the female and male groups (IVF groups 1+2 versus 3+4). The influence of sex was not exhibited by control groups 5 and 6. During the observation period of maximal 7 months anti-HBc seroconversion did not occur in IVF groups. All controls were also anti-HBc-negative after immunization.

DISCUSSION

Hepatitis B immunoglobulin given after exposure to HBV results in prolongation of the incubation period of the virus and partial protection. Beneficial effects of...
postexposure prophylaxis with hepatitis B plasma vaccine have also been demonstrated in adults.9-11

The combination of HBlg and hepatitis B vaccine for postexposure prophylaxis has been shown to increase protection, meaning fewer clinical and HBsAg-positive infections, compared with HBlg or HB-vaccine alone.12-14

Still, although the spouses of acute hepatitis B patients received HBlg (5 ml) on day 1 and plasma vaccine on day 1 and 6 months later (20 μg, Merck Sharp & Dohme), 11% exhibited signs of subclinical infection (anti-HBc-seroconversion) at the 3 month examination.15

Repeated injections of HBlg can, however, in the case of accidental exposure to HBV maintain protective levels of anti-HBs throughout the incubation period.11,15 To accelerate the active immune response, a vaccination schedule with short injection intervals has been proposed for postexposure prophylaxis.16

At the discovery of the HBV epidemic the large majority of patients who had undergone in vitro fertilization were offered postexposure prophylaxis. In order to modify the course of the disease an 'intensive' schedule of two doses of HBlg and four doses of vaccine was offered to the females. To prevent infection in males the standard immunization schedule was used with an additional dose of HBlg at month 1.12 No HBV infections, in particular no anti-HBc-seroconversion, were encountered after the start of passive active immunization, however, the protective effect of passive active immunization in this setting cannot be proven in view of the absence of a control group with comparable exposure. The absence of infections in partners after the start of immunization is remarkable since they may have been exposed to HBV for several additional days or weeks because 34 women were still HBsAg-positive at the start of immunization. Sexual contact is said to be an important mode of transmission.17,18 However sexual exposure appears to be a less efficient mode of transmission compared with the percutaneous route and the chance that men will contract hepatitis B virus during a heterosexual relationship has been estimated to be <5%.12

The overall compliance of couples possibly exposed to HBV appeared to be poor. The loss to follow-up may have been due to lack of motivation since exposure to HBV in IVF treatment period 2 had not occurred (groups 2 and 4) and vaccinees were regularly informed about their immune status.

Passive active immunization of HBsAg-positive or HBV-negative and pregnant vaccinees was practically devoid of side-effects. It is doubtful whether the abortion 2 days after initial immunization was indeed a direct adverse effect of immunization. The first trimester abortion rate after IVF did not differ from that found for the period before HBV contamination of the culture medium was discovered.5 In fact, immunization of pregnant women in case of high risk for hepatitis B infection is advocated. Despite statements of changes in immunity during pregnancy there is little evidence of impairment of cellular immunity in pregnancy. The ability to respond to HBsAg vaccine appeared to be adequate.19

This study provided the opportunity to determine whether administration of HBlg and recombinant HB-vaccine to adults in a high risk situation consistently led to protective levels of anti-HBs soon after immunization. The first dose of HBlg resulted in a protective level of anti-HBs in all but two cases. Subsequent HBlg and vaccine doses did not produce a major increase in anti-HBs levels, except for the booster dose of vaccine at month 6. In fact, administration of a supplementary dose of HBlg at month 4 was thought to be necessary for the majority of the spouses of HBsAg-positive women.

The overall response rate to recombinant HB-vaccine at month 7 was comparable to the response rate for hospital personnel. Protective levels of anti-HBs before the booster dose were, however, relatively low (87% for both female groups and 48% and 81% (mean 63%) for groups 3 and 4, respectively, at month 6), compared with other studies of recombinant vaccine.20-22 In which >93% of healthy adults had anti-HBs ≥10 mIU ml-1 at the time of the booster dose. The 'intensive' immunization schedule with an extra dose of vaccine after 2 months did not appear to accelerate the immune response in females. In fact, as far as postexposure prophylaxis is concerned, immunogenicity after administration of recombinant vaccine was very disappointing in this study.

Recently, Iwarson recommended an accelerated immunization schedule that would yield a more rapid antibody response.16-23 Such a schedule might be an alternative to HBlg in combination with HB-vaccine in the postexposure situation. This study, with a vaccine schedule of 0, 2 and 6 weeks, did not yield satisfactory antibody levels at all, thus emphasizing the need for HBlg in postexposure prophylaxis.

Known factors that influence the immune response to vaccine include age, sex, antigen dose and number of doses given, site of injection and freezing of the vaccine.14-23 Sex-related differences in immune response were found for the combined IVF groups but not for controls. Age, dose, site of injection and vaccine were the same for the IVF groups and controls. Could the large doses of HBlg have interfered with the development of anti-HBs after immunization? Studies of the concurrent administration of HBlg and plasma derived vaccine at one or two occasions showed that the results were similar to those obtained with vaccine alone.26-28 Szmuness et al.28 found that injection of 300 IU of HBlg did not inhibit the anti-HBs response to 20 μg doses of HB-vaccine (Heptavax B, Merck Sharp & Dohme) in adults. In neonates, two major studies have yielded conclusive evidence in favour of the dual approach of postexposure prophylaxis by combination of passive active immunization.3,29 The attack rate for development of the persistent carrier state in neonates of HBsAg-positive HBsAg carrier mothers was 90% in untreated groups. Both HBlg alone or vaccine alone had 70-80% efficacy while efficacy rates in the groups given dual prophylaxis were above 90%.13 There was no statistically significant advantage from multiple doses as opposed to a single dose of immune globulin in conjunction with hepatitis B vaccine for efficacy nor for immune response (GMT).29

Yet, it cannot be excluded with certainty that an inhibiting effect of HBlg was masked by the high dose of the vaccine used in these studies. To investigate this possible interference, Lelie et al.30 compared the anti-HBs response to a low dose (6 μg) of HB inactivated vaccine (CLB, 3 μg per dose) in health-care workers with and without a single dose simultaneously administered HBlg (500 IU). The anti-HBs titres of recipients of vaccine alone compared with those with...
Hepatitis B prophylaxis in IVF women P M Grosheide et al

HB Ig and vaccine were slightly but significantly higher at 3 and 5 months after the first injections but this difference was no longer significant at the time of the booster dose (month 8) and thereafter.

The use of higher doses of HB Ig and the recombinant vaccine with a limited epitope range compared with plasma HBSAg justifies re-examination of a possible interaction between doses of HB Ig and the immune response to recombinant vaccine. Studies of children receiving recombinant vaccine however did not reveal significant differences when supplemental HB Ig was administered.31

Environmental factors have never been known to influence the immune response to immunization. Nevertheless, emotions may have an important effect on the immune system. In rats stress has been shown to suppress cellular and humoral immunity.32,33 For humans in vitro and in vivo studies have demonstrated a direct effect of psychological stress on parameters of immune function.34–36 Several diseases caused by viruses, such as Epstein-Barr virus, herpes simplex virus type I and cytomegalovirus, may be stress-related.37 The ability of human lymphocytes to respond to an activating agent declined significantly within 1—2 months of a serious psychological event. In some people the responses remained low for an entire year.38

Without doubt participation in an in vitro fertilization programme and the subsequent discovery of a possible infection with hepatitis B virus can be considered an episode of stress. For the majority of vaccinees the period of stress lasted until several months after the first injection of vaccine. Therefore the effect of psychological stress on the human immune response may not be ruled out as a possible factor influencing the reactivity to HBSAg.

ACKNOWLEDGEMENTS

The authors are grateful to the nursing staff of the Department of Obstetrics of the Dijkzigt Hospital for their cooperation and the technicians of the Department of Virology for performing the serological assays.

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