Hepatitis B Immunization of Neonates and Infants with a Recombinant and/or Plasma-derived Vaccine in the Giza Area of Saudi Arabia

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Recombinant and/or plasma-derived hepatitis B virus (HBV) vaccine was administered to 157 neonates after delivery and at 1, 2 and 3 months of age in the Giza area of Saudi Arabia. The second, third or fourth dose was received by 59%, 88% or 82% of eligible infants. Anti-HBs responses to two doses of 20 μg recombinant vaccine in 14 infants, one dose of recombinant plus one dose of 5 μg plasma-derived vaccine in 27 infants, and three infants receiving just two doses of plasma-derived lots, were similar. After two or three doses, 34% of 44 infants or 84% of 38 infants, were anti-HBs positive. Infants receiving a subsequent dose or altogether failing to receive such dose(s), did not differ for distance travelled to immunization clinic, sex of the infant or the maternal HBV serologic profile. Only serosurveillance would assist in an effective monitoring of any HBV control plan since HBV exposures in infancy and childhood are mild with a subclinical course. The necessity of boosters could be determined by anti-HBs assays on the first birthday of the vaccinee.

Hepatitis B virus (HBV) infection is acquired very early in life in the Giza area of Saudi Arabia.

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endemicity is attributed to locally practised therapeutic scarification, extraordinary large families, overcrowding and congregation at various functions. These factors operate right from birth, enhance 'horizontal' viral spread and require prophylactic action immediately after birth. Perinatal HBV spread has been effectively prevented in high risk neonates born to HBeAg carrier mothers, by offering recombinant or plasma-derived vaccine at birth and in early infancy. In the developing world, there have been insurmountable problems in the delivery of potent vaccines. Inadvertent freezing or exposure of HBV vaccines to high ambient temperatures has led to poor performance of HBV vaccine in Yucpa Indians in rural western Venezuela. A pilot study has been made for local efficacy of HBV immunization at birth and during infancy among Saudi newborns and infants in the Giza area of Saudi Arabia. HBV markers have been monitored in vaccinees during early infancy.

**Materials and Methods**

**Subjects**

Hepatitis B vaccine was offered to 157 neonates delivered at the King Fahad Central Hospital, Giza area, Saudi Arabia during the period 2 June 1989 to 30 July 1989. The hospital is a referral hospital for the Giza area. Located 1280 km southwest of Riyadh, the hospital serves an area measuring 35,000 km² of Giza area, one of the 14 administrative districts in Saudi Arabia. The population exceeding 500,000 is composed of more than 5000 communities dispersed in towns, villages, farms and at water resources. About one-third of the population is composed of Bedouins who lead a nomadic life-style.

The necessity for HBV vaccination was explained to each mother in Arabic before delivery. Vaccine was offered to neonates, with maternal consent, within 24 h of delivery, in the obstetrics ward of the hospital. Information about the distance between the hospital and the mother’s dwelling site was obtained. Subsequent doses of the vaccine were given in the hospital immunization clinic that operated in the out-patient section. The clinic was managed by a paediatrician, a research physician and an Arabic speaking nurse.

**Vaccination**

Vaccine supplies were received in the regional stores from the Ministry of Health at Riyadh. They were transported by road to the hospital in insulated containers containing cold-packs and stored in the pharmacy cold-room.

Neonates were injected in the anterolateral muscles of the thigh with 1 ml of the recombinant HBV vaccine (Engerix-B, Smith Kline Biologicals) containing 20 μg of antigen. When the supplies of the recombinant vaccine were depleted, 1 ml of plasma derived vaccine (Hevac B, Pasteur) containing 5 μg of antigen, was offered to each neonate or infant.

**Follow-up of vaccinated infants**

Before discharge from the hospital, the mother of every vaccinee was handed a pamphlet, in Arabic, indicating the scheduled date and time, after about one month, for receiving the second dose of the vaccine. During the follow-up visits to the immunization clinic, mothers were informed the next scheduled dates, at monthly intervals, for the third dose to 2-month-old infants and the fourth dose to their 3-month-old infants. Prior to every vaccination in the immunization clinic, an enquiry was made from every mother about any adverse local or systemic reactions attributable to HBV vaccination. No efforts were made to contact the absentees for the second, third or fourth dose at the immunization clinic.

**Blood samples**

Sera from maternal, cord and infant blood samples, were stored frozen, pending testing, at -20 °C.

**Serological testing for viral markers**

Tests for viral serologic markers were made with ELISA kits supplied by Abbott Laboratories. Each serum sample was tested for HbsAg (Auszyme® Monoclonal), anti-HBc (Corzyme®) and anti-HBs (Ausab®). Tests for HBeAg and anti-HBe were made on HbsAg-positive sera with ELISA kit (Hbe rDNA EIA). Alliquots from HbsAg-positive sera were tested for anti-HDV antibody using Abbott anti-HDV kit (Abbott Laboratories, North Chicago, IL).

**Statistics**

For statistical evaluation a χ² test with or without Yates’ correction was used.

**Results**

**Serologic markers in maternal sera**

Serological data were grouped under those positive for HbsAg, anti-HBs and anti-HBc. HbsAg positives included HBsAg positives either exclusively or with anti-HBc and/or anti-HBs, while anti-HBs positives were composed of those additionally positive for anti-HBC. Those exclusively positive for anti-HBE composed the third category. Evidence of a prior HBV infection was present in 40 of the 152 maternal sera tested (Table 1), with respectively positives for HbsAg, anti-HBs and anti-HBC, being 6, 6 and 26. There was no elevation for HbsAg, anti-HBs, anti-HBc or total viral exposure with an increasing age of the mother.

**HBeAg and anti-HBe**

Tests for HBeAg and anti-HBe were made on the six HbsAg-positive mothers. One carrier mother was positive for HBeAg, and four were positive for anti-HBe.

**HDV IgG**

None of the six carrier mothers was positive for HDV IgG.

**Vaccination of neonates**

Vaccine was offered to 160 neonates but maternal consent was received only from 157 mothers. The first dose was Engerix-B in 144 neonates and Hevac-B in 13. Second dose was Engerix-B in 26 cases and 66 received Hevac-B. The third and fourth dose to 81 and 50 infants was of Hevac-B only.

**Follow-up for second or subsequent vaccinations**

The attendance for receiving the second, third and fourth dose was 59%, 88% and 62%, respectively (Table 2). Of the
Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>HBsAg</th>
<th>anti-HBs</th>
<th>anti-HBc</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>28</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>21–30</td>
<td>96</td>
<td>5</td>
<td>2</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>31–40</td>
<td>28</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>152</td>
<td>6</td>
<td>6</td>
<td>28</td>
<td>40</td>
</tr>
</tbody>
</table>

\[
\chi^2 = 1.7, \quad \chi^2 = 5.4, \quad \chi^2 = 4.7
\]

NS: not significant.

Table 2

<table>
<thead>
<tr>
<th>Dose</th>
<th>Eligible</th>
<th>Accepted</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>160</td>
<td>157</td>
<td>98</td>
</tr>
<tr>
<td>Second</td>
<td>157</td>
<td>92</td>
<td>59</td>
</tr>
<tr>
<td>Third</td>
<td>92</td>
<td>81</td>
<td>88</td>
</tr>
<tr>
<td>Fourth</td>
<td>81</td>
<td>50</td>
<td>62</td>
</tr>
</tbody>
</table>

157 neonates who received the first dose, 65 did not report to receive a subsequent dose. The infants receiving their second and later doses were drawn from 28 population sites, while the absentee belonged to 24 population sites, with a solitary infant drawn from outside the area. There was no difference between infants receiving multiple vaccine doses and infants failing to receive any dose in early infancy in respect of the distance to the immunization clinic from the residence, sex of the infant or the maternal HBV serologic profiles (Table 3).

Table 3

Comparison between recipients and absentees for second and subsequent vaccine doses in the immunization clinic

<table>
<thead>
<tr>
<th>Received second and subsequent doses</th>
<th>Received only one dose at birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>92</td>
</tr>
<tr>
<td>Distance from immunization clinic</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>22 km</td>
</tr>
<tr>
<td>SD</td>
<td>22 km</td>
</tr>
<tr>
<td>Range</td>
<td>0.5–105 km</td>
</tr>
<tr>
<td>Sex of infant</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
</tr>
<tr>
<td>HBV markers of mother</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>4/85</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>4/85</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>12/85</td>
</tr>
<tr>
<td>Total HBV exposure</td>
<td>20/85</td>
</tr>
</tbody>
</table>

The prevalence of HBV among

Anti-HBs response among initially seronegatives

Sera from 44 initially anti-HBs negative infants were available after two doses of vaccination and in 38, after three doses. After two doses, 15 (34%) were anti-HBs positive, and after three doses, 32 (84%) were anti-HBs positive. With the third dose, the seroconversion was far better (\(\chi^2 = 18.9; p < 0.001\)). Paired sera were available in 10 infants after the second and third dose. In nine sera with an initial absorbance value < 1.000, there was 1.04–5.3 fold rise in individual absorbance. In one instance with an initial absorbance > 1.000, there was 0.7-fold reduction in absorbance.

Anti-HBs response was available following two doses of Engerix-B or one dose of Engerix-B plus one dose of Hecvac-B in 14 and 27 infants, respectively. With two doses of Engerix-B, six were positive, while with one dose Engerix-B plus Hecvac-B each, only nine responded. None of the three infants receiving two doses of Hecvac-B was positive for anti-HBs.

Discussion

The HBV marker profile in 152 maternal sera in the Gizen area of Saudi Arabia has been examined and no age-dependent rise was found in HBV markers during the child-bearing period. The HBsAg carrier rate of 3.9% (Table 1), with 95% confidence interval (CI) between 0.8 and 7.0% in pregnant females does not differ from an earlier carrier rate of 5% (CI 1.4–8.6%) recorded in 139 pregnant females domiciled in the main township of Gizan. The prevalence for anti-HBs among
pregnant females, 3.9% (CI 0.8–7.0%) is lower than earlier value in Gizan city, 25.2% (CI 18.0–32.4%). Presently we found a larger number of pregnant females only positive for anti-HBc, 18.4% (CI 12.2–24.6%) than 4.3% (CI 0.9–7.7%) in the earlier Gizan city survey. None of the pregnant HBsAg carrier mothers was positive for HDV IgG, though HDV IgG is uniformly distributed in the area among HBsAg carriers, patients with chronic hepatitis and primary hepatocellular carcinoma. The high number of anti-HBc positive females in the present study could justify tests for low-level HBsAg carriage to detect minute amounts of HBV DNA by the polymerase chain reaction.

Both the initial acceptance of HBV vaccine at delivery and during subsequent follow-up in infancy have been encouraging. Before delivery, the attending obstetrician briefed every mother regarding the necessity for immunization. The extent of morbidity and mortality attributable to HBV infection locally were explained. A clinical examination of infants during the follow-up visits to immunization clinic by the attending paediatrician, including a re-briefing by the Arabic speaking nurse about the benefits of re-vaccination and blood sampling, ensured the 88% and 62% compliance to receive the third and fourth dose (Table 2). Maternal consent was obtainable for blood sampling by venepuncture in 82 cases only. The defaulter mothers who did not present the infants for a subsequent vaccine dose would not appear to constitute an atypical subgroup. There has been no difference in respect of distance from the immunization centre, sex of the infant or maternal HBV markers among mothers who had regularly brought infants for a vaccine dose and those who failed to turn up for a second or later vaccine dose (Table 3).

Administration of 380 doses of recombinant or plasma-derived HBV vaccine has been devoid of local or systemic reactions. A similar pattern in infants at large in the area would help in mass acceptability of HBV vaccines. Supplies of recombinant vaccine were exhausted half-way through the study posing a dilemma of administering both recombinant and plasma-derived vaccine to individual neonate or infant, lest the entire study should fizzle out. In retrospect, such a decision about mixing two types of HBV vaccines appears to have been an appropriate one. Sera have been available from 27 infants with one dose of recombinant plus one dose of plasma-derived vaccine, from 14 who received two doses of recombinant and from three who received two doses of plasma-derived vaccine. The anti-HBs response in infants at 2 months of age on any of the three regimens has been similar. In Barcelona, Spain, neonates responded uniformly to recombinant and plasma-derived vaccines when given along with HBV immunoglobulin. Plasma-derived vaccine was given to 25 neonates, while 33 received recombinant vaccine at birth. The respective vaccine lots were repeated at 1 and 2 months of age. With plasma-derived vaccine, there was 88% seroconversion, while 91% vaccines responded to recombinant vaccine lots.

Among the infants who were negative for all HBV markers at birth, seroconversion after two or three doses has been 34% and 84%, respectively. With the third dose, there was a remarkable rise in the number of infants positive for anti-HBs. A rise in anti-HBs level has been demonstrable, albeit indirectly, through a rise in absorbance values, with the third dose among nine of the 15 infants who had seroconverted with two doses of vaccine. Unfortunately the extent of additional seroconversion following the fourth dose in 50 infants has remained unassessed and interesting information has been missed. The extent of additional seroconversion in 16% non-responders to three doses could have as well become available. The suboptimal seroconversions with three doses were effectively tackled by health care workers in Canada by a fourth or fifth vaccine shot. There was a recovery rate of 70% in 45 persons with no or low anti-HBs following three vaccine doses.

The HBsAg is composed of three distinct areas of enveloped gene, the pre-S1 and pre-S2 regions, and the S gene, with each producing a distinct humoral response. Apart from vaccines exclusively with S gene product vaccines containing combined S and pre-S2 gene products have been obtained by genetic recombination in Chinese hamster ovary cells. Three doses of pre-S2 containing vaccines produce anti-HBs more rapidly and with higher titres than have been documented with plasma derived or genetically engineered yeast vaccine. Regular use of pre-S2 containing vaccines singly or in combination with other vaccines might improve the present 84% seroconversions obtained locally by three doses of yeast and/or plasma derived vaccines.

Sera were available from 15 infants who were positive at birth for anti-HBs (12) and anti-HBc (3) for marker testing after two vaccine doses. Among those anti-HBs positive at birth, two exhibited higher anti-HBs absorbance values, while the figures remained unaltered among the remaining 10 infants. Infants with unaltered absorbance figures at 2 months of age cannot be labelled non-responders since the declining maternal antibody level would easily match the ascending infantile antibody, leaving the respective absorbance unaltered. None of the three infants who were positive at birth for anti-HBc have seroconverted for anti-HBs. That is similar to the 34% seroconversion in 44 infants who were anti-HBs negative at birth.
(p = 0.3 Fisher). Any passively acquired anti-HBc would as well exert some protective effect during early infancy. Immunization of chimpanzee with internal viral antigens, HbcAg and HBeAg, do offer substantial or even complete protection against a subsequent viral challenge.12

Hepatitis B virus immune globulin would not be very useful as babies would be protected for 2–4 months only. The uncommon perinatal spread would be tackled during active immunization by pre-S2 containing vaccines. With such vaccines, neonates born to HBeAg-positive mothers produce anti-pre-S2 much earlier than anti-HBs and have the possibility of elimination of viral attack in hepatocytes and any persisting HBV infection.4

A re-assessment for anti-HBs reactivity in all vaccinees is contemplated at their first birthday. Apart from ascertaining the persistence of anti-HBs, it would be desirable to consider the justification of a booster dose for an anamnestic response. Unlike other vaccine-preventable diseases, HBV exposures in infancy and early childhood are mild, run a subclinical course without any illness recognizable as acute hepatitis.1 The success of any HBV control programme can only be monitored through a continuous anti-HBs surveillance of the vaccinees. The ideal period for such an event would be the first birthday and the pre-school entry age of 5 years.

Acknowledgements

We gratefully acknowledge the untiring assistance by Sister Nahed Mohamed Osman, Sister Belinda Villas and Sister Daljeet Walia in this study. We are grateful to the mothers for their co-operation during immunization and consent for venepuncture.

Note added at manuscript revision stage

None of the vaccinees could be bled at the first birthday. Efforts will be made to obtain blood samples for further studies in the near future.

References