Neonatal tetanus despite protective serum antitoxin concentration

S.Y. Maselle 1, R. Matre 3,4, R. Mbise 2 and T. Hofstad 4

1 Department of Microbiology and Immunology, and 2 Department of Paediatrics and Child Health, Muhimbili Medical Centre, Dar es Salaam, Tanzania, 3 Broegelmann Research Laboratory for Microbiology, and 4 Department of Microbiology and Immunology, The Gade Institute, University of Bergen, Norway

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1. SUMMARY

Using the ELISA technique to estimate serum antibodies against tetanus toxin, seven neonates with clinical tetanus were found to have antibody levels 4–13 times higher than the presumed minimum protective level of 0.01 IU/ml. All but one of their mothers had been vaccinated with tetanus toxoid in pregnancy. In two other neonates, whose mothers had received multiple booster doses of toxoid during pregnancy, the anti-toxin concentrations were 100- and 400-times the presumed protective level. Therefore the toxin dose may overwhelm the pre-existing anti-toxin level and produce disease. Furthermore, multiple booster injections of tetanus toxoid may not only enhance serum anti-toxin titres, but could also lead to an ineffective immune response.

2. INTRODUCTION

Many attempts have been made to correlate the amounts of circulating tetanus antitoxin (anti-TT) with the degree of protection in man. However, since human experiments with tetanus are rare, most of the available information is based on animal data which showed that 0.01 international unit (IU) of anti-TT/ml of serum was the threshold of protection [1]. This observation was supported by a human experiment in which 2–3 human minimum lethal doses of tetanus toxin failed to produce symptoms of tetanus with pre-existing 0.005–0.01 IU of anti-TT/ml of serum [2]. Therefore, it has generally been accepted that an anti-TT level of 0.01 IU/ml is the minimum protective level in man [3,4].

However, clinical tetanus has been reported in adult patients with anti-TT concentrations above the presumed minimum protective level at onset of symptoms [5–7]. In Tanzania where neonatal tetanus is an important public health problem, cases have been reported among neonates whose...
mothers were vaccinated with tetanus toxoid (TT) in pregnancy [8,9]. Such reports seemed to suggest that there was probably no universal minimum protective level, and that any serum anti-TT concentration could be overwhelmed given a sufficient quantity of toxin.

This paper describes the findings in ten newborn babies with clinical tetanus seen in a referral to hospital in Tanzania.

3. MATERIALS AND METHODS

3.1. Patients studied

Full term normal delivery newborns with clinical tetanus admitted to Muhimbili Consultant Hospital in Dar es Salaam, Tanzania, were studied. Details of their delivery, onset of symptoms, treatment and history of maternal vaccination with tetanus toxoid (TT) in pregnancy were obtained from their records. Patients who had been given therapeutic anti-tetanus serum were excluded from the study. Blood samples were collected on admission, sera separated and stored at -20°C until analysis.

3.2. Enzyme immuno-assay

Enzyme-linked immunosorbent assay (ELISA) technique was used to determine the anti-TT concentrations in the sera. This technique has been shown to give results comparable with those obtained by in vivo neutralisation test in mice, the conventional method for anti-TT assay [10]. The procedure was essentially similar to that described by Engvall and Perlman [11]. In brief, flat-bottomed polystyrene microtitration plates (Dynatech, Switzerland) were coated with an optimal dilution (see below) of TT ref no. AS/24/81-T containing 480 Lf/ml (National Institute of Public Health (NIPH), Oslo, Norway). Coating was done at 37°C overnight in 0.05 M bicarbonate buffer, pH 9.6. The plates were kept at 4°C and subsequently used for up to 4 weeks.

After washing the plate, phosphate-buffered saline, pH 7.2, containing 1% bovine serum albumin was added to the wells, incubated at 37°C for 1 h and washed. Diluted serum samples were then added, the plate incubated as above and washed again. Thereafter, swine anti-human IgG phosphatase conjugate (Orion Diagnostic, Espo, Finland) appropriately diluted (see below) was added and incubated as above. After further washing, the substrate, p-nitrophenyl phosphate (Sigma Chem. Co., U.S.A.) was added and the plate kept in the dark at room temperature for 20 min. The enzyme-substrate reaction was stopped with sodium hydroxide solution and optical densities read at 405 nm in a Titertek plate reader (Flow Labs, U.K.). Optimal dilutions of TT (1:500) for coating and conjugate (1:500) for use had earlier been established by checkerboard titration with a positive anti-tetanus toxoid control serum (NIPH).

3.3. Calculation of anti-TT concentrations

The optical densities obtained with 14 duplicates of 2-fold dilutions of a positive control serum containing 8 IU of anti-TT/ml (NIPH) were used to draw a standard curve against corresponding serum dilutions. Each patient’s serum had been assayed at three dilutions (1:200, 1:800 and 1:3200) in duplicate and the optical densities obtained were used to determine the anti-TT levels from the standard curve. The final antitoxin concentration for each serum was obtained by averaging any two closest contiguous estimations. A negative control serum from an unvaccinated person and a positive control were included in all assays. One person performed the assays and there were no changes in reagents’ lots or equipment.

4. RESULTS

4.1. Patients studied

Ten neonates with tetanus fulfilled the study criteria. Five of them had been delivered at home and the other five in hospital or maternity homes. Three of the respective mothers were primiparous and the remaining seven were multiparous. All the babies had clinically infected umbilical cord stumps, except one (case no. 10). One patient had been treated with crystalline penicillin and another, who was delivered at home, had local treatment with a mixture of charcoal ash and kerosine. The rest had not received any form of treatment.
Table 1

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Incubation period in days</th>
<th>Maternal TT vaccination</th>
<th>Serum anti-TT concentration</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>Previous pregnancies</td>
<td>Last pregnancy</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>No (p)</td>
<td>2 doses</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>No (p)</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>5 doses</td>
<td>1 dose</td>
</tr>
<tr>
<td>4</td>
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<td>3 doses</td>
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<tr>
<td>10</td>
<td>14</td>
<td>No</td>
<td>1 dose</td>
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</tbody>
</table>

p. primiparous; ns, not specified.

Table 1 shows the incubation periods, maternal TT vaccination status and serum anti-TT concentrations in the ten patients. The incubation periods calculated from the day of delivery up to the onset of symptoms varied from 2–16 days with a mean of 7.9 days. Nine mothers had been vaccinated with TT in the last pregnancy, five of whom had TT in previous pregnancies. One mother had not received any TT vaccination. There was no relationship between the incubation period and maternal vaccination status or anti-TT levels.

4.2. Anti-TT levels in the patients’ sera

Anti-TT serum levels in the patients shown in Table 1 were all above 0.01 IU/ml except in one patient (no. 10) who had a titre of 0.002 IU/ml. The mother of this patient had been given a single dose of TT 2 weeks before delivery. She had not been vaccinated before. The highest anti-TT level of 4.220 IU/ml was recorded in patient no. 4 whose mother had received multiple inoculations in each of two consecutive pregnancies over a 3-year period. The second highest level of 1.035 IU/ml was in patient no. 6 whose mother had received 14 doses of TT in 5 consecutive pregnancies over a 13-year period. This mother had been given 2–3 doses of toxoid during each of the 5 pregnancies. One patient (no. 2) whose mother was not vaccinated had a serum anti-toxin level of 0.037 IU/ml.

4.3. Anti-TT levels and maternal vaccination

Table 2 shows the relationship between mean anti-TT concentrations and the number of TT doses given in the last pregnancy. There was considerable individual variation in the anti-toxin levels as shown by the wide standard deviations and coefficients of variation within the groups. There was a general trend for anti-TT levels to be higher with increasing number of TT doses. However, the number of patients studied was too small for any statistical analysis.

5. DISCUSSION

This study was undertaken because despite an intensive programme of TT administration in pregnancy and training of birth attendants in hygienic practices, there are still problems with tetanus neonatorum in Tanzania. Although the incidence has fallen from 2% per live birth in 1979 [8] to 0.6% in 1989, it is estimated that 40% of the cases occur in neonates whose mothers had TT in pregnancy [8]. This was not due to vaccine failure [12], but rather to presumed effective levels of anti-toxin failing to prevent tetanus. This is the first report documenting the occurrence of “protective” levels of anti-toxin in neonates with tetanus.

By using the ELISA technique to determine specific IgG antibodies against tetanus toxin, non-neutralising antibodies could have been included in the estimations as it was not possible to
know how much of the absorbency was due to such antibodies. However, previous studies have shown that the technique gives results comparable with those obtained using methods which assay neutralising antibodies [9]. It is likely, therefore, that neonatal tetanus occurred with pre-existing toxin-neutralising antibodies above the presumed minimum protective level of 0.01 IU/ml.

There are previous reports of adult patients with tetanus in whom neutralising antibodies assayed using the animal protection model [5–7], were present. In these reports a total of 12 patients had anti-TT concentrations ranging from 4–16-times the protective level. In our study, seven patients had anti-TT concentrations 4–13-times the minimum protective level. These observations suggest there is no absolute ‘minimum protective level’ of tetanus anti-toxin. This may be particularly important in neonates who rely on passively acquired immunity; the level of 0.01 IU/ml in maternal serum may not provide reliable protection. The toxin dose in relation to the level of neutralising antibodies probably determines whether tetanus develops.

Two of our patients (nos. 4 and 6) had anti-toxin concentrations 400- and 100-times the minimum protective level, respectively. These are the highest reported pre-existing anti-toxin levels in patients with tetanus, and an explanation is required. The mothers of the two patients had been given multiple booster doses of TT in successive pregnancies which might induce predominantly IgG4-restricted response [13]. Antibodies of the IgG4 subclass are functionally monovalent [14], and may be of low affinity in man [15]. We did not determine whether the anti-TT in our patients was IgG4 isotype, but these observations could provide an explanation for the development of tetanus in our patients. If this explanation is correct, repeated use of TT in pregnancy is undesirable because it may not be protective as well as frequently causing allergic reactions [12].

The presence of 0.037 IU/ml in patient no. 2, whose mother had not been vaccinated, is more difficult to explain, but could have been due to childhood immunization, although naturally acquired antibodies to tetanus toxin in man have been reported [16]. Serum samples are rarely obtained in patients with clinical tetanus, and we have found only three reports [5–7].

In conclusion, the programme to prevent tetanus neonatorum should avoid hyperimmunisation of women. In addition to the immunisation programme education and training in hygiene remain vitally important.

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REFERENCES


