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Timing of antenatal tetanus immunization for effective protection of the neonate

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A study was undertaken to determine the relationship between the timing of maternal immunization with a commercially available adsorbed tetanus toxoid and the presence of protective antitoxin in cord blood. Women at various stages of gestation were given one or two doses of 20 IU of toxoid, and the maternal and cord sera collected at delivery were assayed for tetanus antitoxin by the indirect haemagglutination and toxin neutralization techniques.

Results indicated that the first injection of a two-dose schedule should be given at least 60 days, and preferably 90 days or more before delivery, with the second injection 20 days or more before delivery. The single-dose schedule conferred no significant protection when given less than 70 days before delivery; beyond 70 days, protection rates improved, but there were too few subjects to allow any definite conclusions to be made.

Cord and maternal antitoxin titres differed by no more than one twofold dilution for almost all of the individual paired sera. A cord/maternal antitoxin ratio of 2 was more likely to occur with increasing time between the second injection and delivery.

The incidence of neonatal tetanus in many parts of the world is unknown, but Bytchenko (*1*) has estimated that, in some areas, tetanus neonatorum accounts for 10–30% of all cases of tetanus. Furthermore, the case-fatality rate of tetanus in newborns has been reported as ranging from 20% to 100% (*1*). According to Miller (*2*), tetanus during the first month of life accounts for 25–50% of deaths from tetanus throughout the world. Even with modern methods of treatment, the case-fatality rate is considerable; for example, a rate of 20.6% was reported from the University Hospital, Kuala Lumpur, Malaysia by Chen in 1974 (*3*). The neonate, then, is particularly in need of protection against tetanus.

It is well documented that the active immunization of women, during or before pregnancy, can prevent tetanus neonatorum through the transplacental passage of maternal antitoxin. It has been shown that, while two or three suitably timed injections of plain tetanus toxoid in pregnant women are capable of preventing or significantly reducing neonatal tetanus (*4*), the use of adsorbed rather than fluid toxoid is more effective in providing the generally accepted protective titre of 0.01 IU of tetanus antitoxin per ml of cord blood (*5*, *6*). MacLennan et al. (*7*) confirmed the superiority of adsorbed over plain toxoids in terms of persistence of the antitoxin formed. Toxoid with oil adjuvant gave even more persistent titres in this study, but also had unacceptable side-effects. In addition, the study showed that a maternal antitoxin titre of 0.01 IU/ml at delivery is protective for the neonate.

In a double-blind, controlled field trial, Newell et al. (*8*) demonstrated that immunization of all women of childbearing age with two or three doses of adsorbed toxoid can eliminate neonatal tetanus from a population for up to five years. Stanfield et al. (*9*) examined the efficacy of two-dose and one-dose schedules of various toxoids and adjuvants in producing protective antitoxin titres in pregnant women at delivery, with the ultimate goal of providing an effective one-dose schedule. The best single-dose toxoid tested was 100 Lf of an adsorbed toxoid containing a quaternary ammonium adjuvant, which produced protective levels of antitoxin in 83% of the mothers when given at least 60 days before delivery. This group also reported that the transfer of antitoxin from mother to fetus was more effective and rapid after two doses of toxoid than after one dose. Subsequently, the effect of varying the interval between

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administration of two doses of adsorbed toxoid to pregnant women was investigated (*10*). An analysis of cord blood titres indicated that the two injections should be given not less than 12 weeks apart, with the second at least 4 weeks before delivery, but it was conceded that this may not be feasible during pregnancy.

Despite its proven efficacy, mass immunization of women of childbearing age is not possible in many places, and the use of single-dose, high-potency toxoids, although promising, is not yet generally applicable. Immunization on first attendance at an antenatal clinic is still widely practised. Under these circumstances, presentation in late pregnancy and the limited time available may restrict the choice of timing of injections and the interval between injections. Hence, there remains a need to define the time factors limiting the effectiveness of a conventional two-dose schedule of generally available toxoids. We undertook such a study in the Obstetrical Unit of the University Hospital, University of Malaya.

The objective of the study was to determine the relationship between the timing of administration of tetanus toxoid to pregnant women and the presence of protective titres of antitoxin in the cord blood at delivery. Specifically, it was hoped to determine the length of time before delivery that the first of a two-dose schedule of a commercially available tetanus toxoid should be given—with the second dose at the usual interval of about six weeks later—in order to provide a reasonable probability of achieving a protective titre of antitoxin in the cord blood. We also examined cord blood antitoxin titres after the administration of one dose of toxoid at various stages of gestation, as well as the cord/maternal blood antitoxin ratio and its relationship to time between injection and delivery.

MATERIALS AND METHODS

Selection of subjects and immunization schedules

All new admissions to the Obstetrical Service of the University Hospital, Kuala Lumpur, were offered immunization against tetanus provided they were at least 16 weeks pregnant and had no history of previous tetanus immunization. Two doses of tetanus toxoid were usually administered to those who gave their free and informed consent, but some received only one dose. Each dose of 0.5 ml contained 20 IU of tetanus toxoid, adsorbed with aluminium hydroxide.[a]

The first dose of toxoid was given between 16 and 39 weeks of gestation, with a median of 29 weeks. The preferred interval between doses was 6 weeks for those first inoculated before the 32nd week, and 4 weeks for those inoculated after the 32nd week of pregnancy. The actual intervals ranged from 3 to 20 weeks with a median of 6 weeks.

Toxoid was not given if the patient had acute fever, serious complications of pregnancy, malignancy, or was being treated with steroids or immunosuppressive drugs.

Blood sampling and titration

Where possible, a pre-immunization blood sample was drawn from the mother. At delivery, a 5-ml sample of cord blood was collected; within 24 hours of delivery, 5 ml of blood was collected from the mother. Each blood sample was labelled with the registration number, initials or name of subject, date of collection, and source (cord or mother). The blood was centrifuged and the serum transferred to a labelled, screw-capped vial within 24 hours. Batches of vials were packed in dry ice and transported by air to the London School of Hygiene and Tropical Medicine, where they were stored at -20 °C pending titration for tetanus antitoxin.

Sera were titrated initially using an indirect haemagglutination technique (*11*) to identify those with no antitoxin and to obtain an antitoxin level for the remainder in haemagglutination units per ml. These values were then used as a guide to reduce the number of twofold serum dilutions used in the toxin neutralization test (*12*). After a number of antisera had been assayed, it became apparent that the toxin neutralization titres for paired cord and maternal samples rarely differed by more than one twofold dilution. Antitoxin titrations by indirect haemagglutination were subsequently restricted to cord sera. Adoption of this procedure obviated the need for prior absorption of the sera by sheep cells as cord sera do not possess heterophile agglutinins for sheep cells (*13*).

The toxin neutralization assays were usually conducted at the L+/4000 or L+/100 toxin test dose level using one mouse per dilution over a range of 5 twofold dilutions. The minimum titre of tetanus antitoxin so determined was 0.0025 IU/ml.

Characteristics of the study group

Data were recorded on computer cards for each subject, regarding ethnic group, age, parity, household income, history of serious disease, normalcy of delivery, sex of baby, congenital abnormalities, gestation at time of delivery, Apgar recovery score at 5 minutes, dates of administration of first and second doses of toxoid, lot number of toxoid, side-effects of immunization, interval between first and second

[a] Lot numbers 058618, 80944, 81027, 81029, 81067, 81069, 82346, 82374, 82376, 85953, 88474. From Wellcome Research Laboratories, Langley Court, Beckenham, Kent, England.

injections, intervals from first and second injections to delivery, and antitoxin titres of cord and maternal sera.

The study group comprised 44% Chinese, 30% Malay, 24% Indian, and 2% other ethnic groups. Ages ranged from 14 to 42 years with a median of 25 years. Parity ranged from 1 to 8 with a median of 2. Monthly incomes were divided into groups of M$ 100, ranging from M$ 0–99 to M$ 500 and above; the median monthly income was M$ 300–399.

Three babies were born with serious congenital abnormalities, giving an incidence of 0.8%, which is similar to the overall 0.9% incidence of such abnormalities at the University Hospital. The incidence of prematurity among this group of mothers was 6% which was not significantly different from the 7% overall incidence seen at the University Hospital. Altogether, 94% of the babies had an Apgar recovery score of 9–10 at 5 minutes.

RESULTS

Selection of titration results

Of the 101 subjects who gave blood samples before immunization, 16% were found to have a positive titre (> 0.0025 IU/ml) indicating prior tetanus immunization despite the negative history. In view of this finding, the post-immunization titration results from the larger group without pre-immunization determinations were carefully selected for analysis. Samples with a titre of 6.4 IU/ml or more were rejected on the basis that such high titres could not with certainty be attributed to a two-dose immunization schedule with toxoids of normal potency. This selection process resulted in the rejection of 19.7% of the samples, an even greater proportion than the 16% found positive among the pre-immunization samples. For the one-dose schedule, titres of more than 0.01 IU/ml up to 29 days after injection were rejected, as such titres are not usually produced so soon after a single dose of tetanus toxoid of normal potency (*14*). Titres of 1.6 IU/ml or more at any time after the single injection were also disregarded. By these means, 15% of the titration results for the one-dose immunization schedule were rejected.

Group characteristics

Ethnic group, age, parity, and household income had no significant influence on the percentages of samples of cord blood with protective titres. The incidence of side-effects of immunization was low: about 2% of the subjects reported mild localized reactions or fever.

Two-dose immunization schedule

The relationship between the time of first injection of the mother and the percentage of samples of cord blood with 0.01 IU/ml or more is shown in Table 1 for the 49 subjects with negative pre-immunization titres and in Table 2 for all 167 subjects. The pattern was similar for the two groups, in that protection was conferred on 80% or more of the newborns whose mothers received their first injection 60 days or more before delivery. Protective levels of antitoxin were seen in all cord blood samples from infants whose mothers had received their first injection 90 days before delivery. However, even when the period from the first injection to delivery exceeded 90 days, a small number of cord sera had titres of < 0.01 IU/ml—one each in the groups with intervals of 100–109 days ($\leqslant 0.0025$ IU/ml), 110–119 days ($\leqslant 0.0025$ IU/ml), and 140–149 days (0.005 IU/ml). The mother of the first of these received her second injection only 3 days before delivery which is too short a time for the development of a secondary response and passage of the antitoxin to the baby, but the other two mothers received their second injections 84 and 99 days before delivery, and may be regarded, therefore, as non- or low-responders.

For the calculation of the geometric mean titres given in Table 2, titres $\leqslant 0.0025$ IU/ml were assumed

Table 1. Percentage of cord blood samples with a protective titre of tetanus antitoxin, according to interval between first injection and delivery, for 49 subjects with negative pre-immunization titres: two-dose schedule

Interval between first injection and delivery (days)	No. of subjects	Cord blood samples with titre $\geqslant 0.01$ IU/ml (%)
20–29	1	0
30–39	4	25
40–49	5	40
50–59	7	57
60–69	4	100
70–79	7	100
80–89	2	100
90–99	2	100
100–109	0	–
110–119	3	67
120–129	2	100
130–139	5	100
140–149	3	100
150–159	3	100
160–169	1	100

Table 2. Percentage of cord blood samples with a protective titre of tetanus antitoxin, according to interval between first injection and delivery, for all 167 subjects: two-dose schedule

Interval between first injection and delivery (days)	No. of subjects	Cord blood samples with titre ⩾ 0.01 IU/ml (%)	Geometric mean titre (IU/ml)
20–29	3	0	0.001
30–39	13	23	0.004
40–49	12	25	0.004
50–59	25	76	0.04
60–69	15	80	0.03
70–79	19	79	0.11
80–89	16	88	0.10
90–99	14	100	0.34
100–109	10	90	0.21
110–119	5	80	0.21
120–129	12	100	0.24
130–139	8	100	0.49
140–149	7	86	0.33
150–159	3	100	0.58
160–169	3	100	0.19
170–179	0	–	–
180–189	2	100	0.08

Table 3. Percentage of cord blood samples with a protective titre of tetanus antitoxin, according to interval between second injection and delivery, for 49 subjects with negative pre-immunization titres

Interval between second injection and delivery (days)	No. of subjects	Cord blood samples with titre ⩾ 0.01 IU/ml (%)
0–9	7	29
10–19	6	67
20–29	9	100
30–39	9	67
40–49	6	100
50–59	2	100
60–69	2	100
70–79	2	100
80–89	3	67
90–99	0	–
100–109	0	–
110–119	1	100
120–129	2	100

to have a value of 0.00125 IU/ml (*15*). Although there was no significant difference between the percentages of cord blood samples with titres ⩾ 0.01 IU/ml for intervals of 60–69 and 70–79 days, there was a difference of severalfold magnitude in the geometric mean titres for these two intervals. This reflects the fact that one-third (4 of 12) of positive sera from the 60–69-day interval had a low positive titre of 0.01 IU/ml, whereas only 1 of 15 positive sera from the 70–79-day group had this titre.

Tables 3 and 4 show the relationship between interval from second injection to delivery and the percentage of cord blood samples with protective antitoxin titres, for the 49 subjects with negative pre-immunization titres and for all 167 subjects in the two-dose programme. Once again, the patterns for the two groups were similar, with no significant degree of protection when immunization was carried out less than 20 days from delivery. In the larger series (Table 4), protective titres were found in all cord blood samples when the second maternal injection was given 60 days or more before delivery, except for the previously described non-responder in the 80–89-day group and the low-responder in the 90–99-day group.

A total of 29 mothers (17%) received their second dose of toxoid less than 10 days from delivery; three

Table 4. Percentage of cord blood samples with a protective titre of tetanus antitoxin, according to interval between second injection and delivery, for all 167 subjects

Interval between second injection and delivery (days)	No. of subjects	Cord blood samples with titre ⩾ 0.01 IU/ml (%)
0–9	29	28
10–19	26	69
20–29	28	89
30–39	23	87
40–49	19	95
50–59	12	83
60–69	10	100
70–79	5	100
80–89	4	75
90–99	2	50
100–109	2	100
110–119	2	100
120–129	3	100
130–139	1	100
140–149	1	100

were immunized only 1 day before delivery and one 2 days before delivery. Of the cord blood samples from this group, 21 had titres of less than 0.01 IU/ml.

One-dose immunization schedule

Only 12 (17%) of the cord blood samples from the 70 subjects who received a single dose of toxoid had a protective antitoxin titre of 0.01 IU/ml or more. The geometric mean titre of these 12 samples was 0.04 IU/ml. Six were from the 10 mothers who had received the injection 70 days or more before delivery, giving a protection rate of 60%; among the remaining 60 mothers immunized less than 70 days before delivery, the protection rate was only 10%.

Cord/maternal blood antitoxin ratios

Where maternal as well as cord blood was assayed, the cord/maternal blood antitoxin (C/M) ratios were determined (Table 5). In only 2 of the 64 paired sera did the cord and maternal antitoxin titres differ by more than a single twofold dilution. A C/M ratio of 0.125 occurred in one subject who had received her second injection only 8 days before delivery.

Table 6 shows the number of days from first and second injections to delivery for the paired sera from the two-dose immunization schedule with C/M ratios of 0.5 and 2.0. An analysis of variance of these data revealed a significant difference in the interval between second injection and delivery for the two groups ($P < 0.01$).

Table 5. Cord/maternal blood antitoxin (C/M) ratios for paired sera, assayed by the toxin neutralization test using a series of twofold dilutions

C/M ratio	No. of subjects		Total
	Two-dose schedule	One-dose schedule	
0.125	1		1
0.25	1		1
0.5	21	3	24
1.0	19		19
2.0	18	1	19
Total			64

Table 6. Intervals (mean ± S.D.) between injections and delivery for cord/maternal blood antitoxin (C/M) ratios of 0.5 and 2.0: two-dose schedule

C/M ratio	No. of subjects	Time before delivery (days)	
		First injection	Second injection
0.5	21	87.95 ± 33.28	31.76 ± 22.22[a]
2.0	18	97.28 ± 33.21	58.39 ± 35.67[a]

[a] Significant difference ($P < 0.01$).

DISCUSSION

Defining an "acceptable" level of protection in terms of percentage of cord blood samples with protective antitoxin titres presents some difficulties. A 100% protection rate may be too stringent a requirement and is, perhaps, unrealistic in a population in a tropical environment where the incidence of low- or non-responders may be higher than elsewhere. It has been shown, for example, that the presence of blood parasites can depress the immune response to tetanus toxoid in human subjects (*16*, *17*) and mice (*18*). No information was available on parasitaemia in the study group. However, there did appear to be one low- and one non-responder. A further complication is that the actual protective titre of tetanus antitoxin may be less than the generally accepted level of 0.01 IU/ml (*15*). Stanfield et al. (*9*) considered that maternal protection rates of 73% and 83%, although not generally regarded as acceptable, may be "approaching acceptable rates", since one-third of the mothers with less than 0.01 IU/ml of antitoxin could be shown to possess some antitoxin which would, therefore, offer some protection to their infants.

The use of maternal protection rates in assessing the efficacy of tetanus immunization in preventing neonatal tetanus is based on an extrapolation of the observation of MacLennan et al. (*7*) that no tetanus neonatorum occurred in five babies with titres less than 0.01 IU/ml born to mothers with protective titres. In our study, however, the effects of timing of maternal immunization on cord blood titre was measured directly on samples of cord blood. Results showed that the first dose of a two-dose schedule of toxoid should be given at least 60 days before delivery in order to provide a reasonable chance (80%) of conferring protection on the neonate. The probability of protection increases as the time between first injection and delivery increases beyond 60 days. Acceptable levels of protection were associated with administration of the second dose at least 20 days before delivery.

Results of the single-dose schedule indicated that the protection conferred up to 70 days after immunization is insignificant. Beyond 70 days, the probability

of protection apparently increases, but there were too few subjects in this category to give reliable indications. Nevertheless, the percentage of cord blood samples with a protective titre associated with immunization 70 days or more before delivery (60%) is similar to the 70% of maternal sera found by Stanfield et al. (*9*) to have protective titres at delivery when injections of 20 Lf of adsorbed toxoid were given at least 60 days before delivery. In that study, too, there were only 10 subjects.

The examination of cord/maternal antitoxin ratios was of particular interest because ours is the first study to use a toxin neutralization test with twofold dilutions of sera (*12*). The findings showed that the difference in antitoxin titres between cord and maternal sera was no more than a single twofold dilution for almost all pairs. The longer the time from second injection to delivery, the greater the likelihood of the cord titre exceeding the maternal titre. This is in line with earlier observations (*9*) that the transfer of antitoxin is more efficient and rapid after the second injection of toxoid than it is after the first; however, with regard to the timing of the second injection, this effect must be balanced against the increase in titre that results from a longer interval between toxoid injections (*10*).

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RÉSUMÉ

CALENDRIER OPTIMAL DE LA VACCINATION ANTITÉTANIQUE PRÉNATALE DE LA MÈRE EN VUE D'ASSURER LA PROTECTION DU NOUVEAU-NÉ

L'objet de la présente étude était de déterminer les rapports entre le calendrier des injections d'anatoxine tétanique à la femme enceinte et l'existence, lors de l'accouchement, d'un taux de protection acceptable au niveau du sérum du cordon ombilical.

Des groupes de femmes enceintes se trouvant à un stade plus ou moins avancé de leur grossesse ont reçu une ou deux doses de 20 UI d'anatoxine tétanique adsorbée. Dans le second cas, les deux doses étaient administrées à intervalle de 3 à 20 semaines, avec un intervalle médian de 6 semaines.

Des prélèvements de sang ont été pratiqués au moment de l'accouchement, à la fois chez la mère et au niveau du cordon, et l'on a mesuré la teneur en antitoxine tétanique par des techniques d'hémagglutination indirecte et de neutralisation de la toxine. L'analyse d'une série de sérums prélevés avant la vaccination a montré que 16% d'entre eux contenaient de l'antitoxine tétanique malgré l'absence de toute vaccination antitétanique antérieure. Devant ce résultat, on a décidé d'exclure de l'étude tous les titres supérieurs ou égaux à 6,4 UI/ml dans le cas du schéma à double dose, vu qu'il était impossible d'attribuer avec certitude des titres aussi élevés à l'administration de deux doses d'anatoxine d'activité normale; de ce fait, 19,7% des résultats du titrage ont été rejetés. Ont également été exclus les titres dépassant 0,01 UI/ml jusqu'au 29^{e} jour après une injection unique et les titres supérieurs ou égaux à 1,6 UI/ml à un moment quelconque après cette injection, ce qui a entraîné l'élimination de 15% des résultats du titrage pour le schéma vaccinal à une seule dose.

L'analyse des cas restants et des cas comportant un titre nul avant la vaccination a montré que, lorsque la première dose est administrée au moins 60 jours avant l'accouchement, on observe dans 80% des cas un titre d'antitoxine protectrice d'au moins 0,01 UI/ml dans le sérum ombilical. A 90 jours, le taux de protection était de 100%. Dans ces conditions, la première injection d'une anatoxine tétanique d'activité normale doit se faire, dans le cas d'un schéma à deux doses, au moins 60 jours, et de préférence 90 jours, avant l'accouchement. Quant à la seconde dose, elle doit être administrée au moins 20 jours avant l'accouchement.

Même lorsque la première injection avait précédé l'accouchement de plus de 90 jours, on a observé, dans trois cas, un titre d'antitoxine inférieur à 0,01 UI/ml au niveau du cordon ombilical. Dans deux de ces cas, on peut estimer qu'il s'agissait de sujets présentant une réaction faible ou nulle, mais, dans le troisième cas, l'intéressée avait reçu la seconde injection trois jours seulement avant d'accoucher, délai trop court pour permettre une réponse secondaire et le passage de l'antitoxine dans le placenta.

Avec le schéma à dose unique, le taux de protection n'a été que de 10% quand l'injection n'avait été faite que moins de 70 jours avant l'accouchement. Ce taux est passé à 60% (6 femmes sur 10) quand l'injection avait été pratiquée au moins 70 jours avant l'accouchement, mais les effectifs en cause sont trop réduits pour qu'on puisse en tirer des conclusions définitives.

L'écart entre les titres de l'antitoxine dans le sérum maternel et dans le sérum du cordon ombilical s'est révélé supérieur à une dilution (dilutions pratiquées de 2 en 2) dans

deux paires de sérums seulement sur 64; et dans l'un de ces deux cas, la seconde injection n'avait été pratiquée que 8 jours avant l'accouchement. Les observations réalisées ont également montré que plus la seconde injection est pratiquée longtemps avant l'accouchement, plus le titre observé au niveau du cordon a de chances d'être supérieur au titre du sérum maternel. Cette constatation recoupe des observations antérieures selon lesquelles le passage de l'antitoxine est plus efficace et rapide après la seconde injection qu'après la première. Toutefois, il faut apprécier cet effet compte tenu du fait qu'il est établi que le titre augmente en même temps que la durée séparant les deux injections d'anatoxine.

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