Neonatal Tetanus in Peru: Risk Assessment with Modified Enzyme-Linked Immunosorbent Assay and Toxoid Skin Test

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ABSTRACT

We used a modified enzyme-linked immunosorbent assay (ELISA) to investigate tetanus immunity in 232 pregnant Peruvian women. One hundred forty-two (61.2%) had protective antitoxin titers (≥0.01 IU/mL). Protective titers correlated positively with the number of toxoid doses reported during the current pregnancy. A majority of women reporting no toxoid doses during the current pregnancy had at least one prenatal health care visit. We evaluated a toxoid skin test in 44 of the subjects, but it correlated poorly with the ELISA. The modified ELISA is a useful in vitro method for studying tetanus immunity in the developing world. (*Am J Public Health.* 1993;83:1754–1756)

Introduction

Neonatal tetanus causes over 780 000 deaths worldwide each year.[1] Peru reports approximately 0.4 cases per 1000 live births, one of the highest rates in Latin America.[2] Yet adequate maternal immunization with tetanus toxoid can completely prevent the disease.[3,4] During pregnancy, two adequately spaced injections of tetanus toxoid induce protective levels of antitoxin in more than 80% of previously unimmunized women, and three injections protect virtually 100%.[5,6]

Antitetanus vaccination efforts in the Third World have failed to reach large numbers of women of childbearing age. No information on the success of such efforts in Peru has been published.

Serologic investigation of tetanus immunity in the developing world presents several problems. The gold standard mouse neutralization assay is costly and cumbersome. Routine use of the enzyme-linked immunosorbent assay (ELISA) and hemagglutination have proven unreliable for measuring low titers.[7,8] Simonsen et al. have developed a modified competitive ELISA that correlates extremely well with the in vivo standard.[9] This recently developed in vitro assay has not been used to study tetanus immunity in a Third World population.

Even with the modified ELISA, large-scale studies of tetanus immunity in the developing world remain difficult. Intradermal injections of tetanus toxoid elicit a delayed hypersensitivity reaction in a large percentage of previously immunized subjects, and this reaction may correlate with serum antitoxin titer.[10,11] A reliable skin test would greatly facilitate the study of tetanus immunity.

We designed a study to assess serum tetanus antitoxin levels in pregnant Peruvian women with the modified ELISA. We administered a tetanus toxoid skin test to a subset of our subjects to investigate its use as a screen for tetanus immunity.

Subjects and Methods

Subjects

Subjects were pregnant women presenting in labor to three hospitals: Cayetano Heredia, serving a poor and lower-middle-class population in Lima; Maternidad de Lima, a large maternity hospital serving poor women from Lima and its shantytowns; and Hospital Regional del Amazonas in the city of Iquitos in the Amazon jungle.

Women admitted in labor to these hospitals during the study period were enrolled. Verbal consent was obtained. Subjects were interviewed about antitetanus vaccination during their current pregnancy. A blood specimen was obtained by venipuncture. A tetanus toxoid skin test was performed on a subset of the subjects.

ELISA

The modified ELISA was performed according to Simonsen et al.[9]

Skin Test

A 0.1-mL dose of vaccine-grade adsorbed tetanus toxoid (Connaught) was injected intradermally on the anterior aspect of the forearm. Reactions were scored as the greatest diameter of induration at 36 to 72 hours. A positive cutoff of 5 mm or more was chosen.

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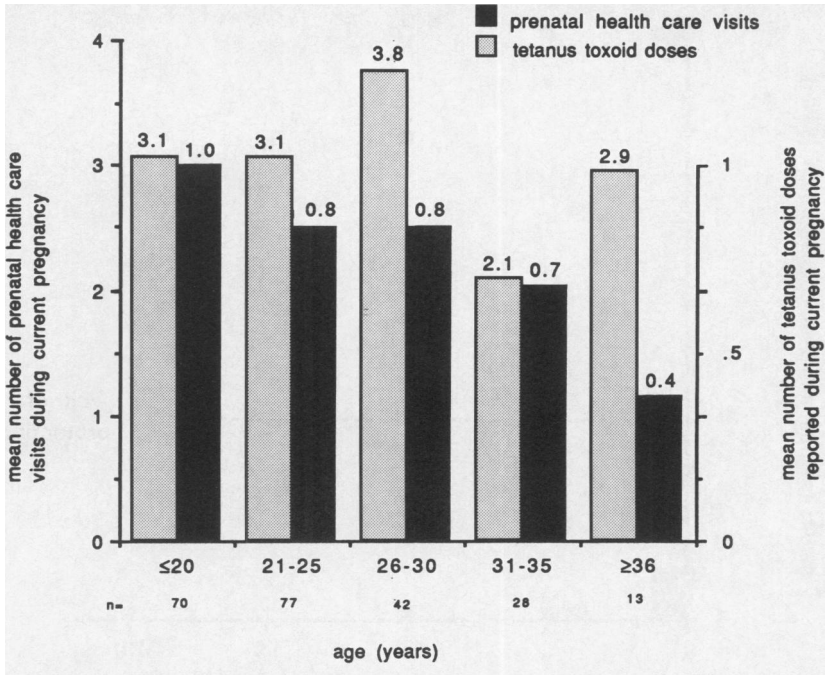
TABLE 1—Percentage of Subjects with Protective Serum Tetanus Antitoxin Levels, by Hospital

Hospital (Location)	No. Subjects	Subjects with Protective Antitoxin Level (≥ 0.01 IU/mL),[a] %
Maternidad (capital)	141	58.2
Cayetano (capital)	21	71.4
Amazonas (jungle)	70	64.3
Total	232	61.2

[a] $P = 0.42$, chi-square test, for comparison among hospitals.



Note. n = 230 because prenatal health care data were not available for two subjects. Prenatal healthcare visits, $P = .69$, linear regression analysis; tetanus toxoid doses, $P = .027$, linear regression analysis.

FIGURE 1—Mean number of prenatal health care visits and mean number of tetanus toxoid doses reported during the current pregnancy, by age group.

Results

A total of 232 pregnant women in labor (mean age ± SD = 24.5 ± 5.9 years) were included in the study. The number of pregnancies (including the current one) ranged from 1 to 10 (median = 2; 10th and 90th percentiles = 1 and 6). Subjects reported a mean of 3.1 (SD = 3.0) prenatal health visits and a mean of 0.9 (SD = 0.9, range = 0–3) tetanus toxoid injections during the current pregnancy.

A total of 142 of 232 (61.2%) subjects had a serum tetanus antitoxin concentration above the protective level of 0.01 IU/mL. No difference was found among hospitals ($P = .42$, chi-square test; Table 1).

Of the subjects reporting 0, 1, 2, and 3 tetanus toxoid injections during the index pregnancy, 39.6%, 71.7%, 87.3%, and 100%, respectively, had a protective serum antitoxin level ($P < .00001$, chi-square test for linear trend). Both the percentage of subjects with positive titers and the number of tetanus toxoid doses reported during the pregnancy decreased with increasing age group ($P = .00036$, chi-square test for linear trend [positive titers]; $P = .027$, linear regression analysis [tetanus toxoid doses]), but we found no relationship between age and the number of prenatal health care visits ($P = .69$, linear regression analysis; Figure 1).

Of the subjects who reported receiving no tetanus toxoid injections during the pregnancy, 55 of 106 (51.9%) gave a history of at least one prenatal health care visit and 49 of 106 (46.2%) gave a history of two or more such visits. Of those reporting only one injection, 39 of 60 (65.0%) reported at least two prenatal health care visits.

Tetanus toxoid skin tests were administered to 70 subjects. Twenty-six tests (37.1%) were not read because the subject was discharged less than 36 hours after placement. Of the 44 skin tests read, 18 (40.9%) were positive, but the test correlated poorly with the modified ELISA, with a sensitivity of 57.7% and a specificity of 83.3% (Figure 2).

Discussion

The modified ELISA developed by Simonsen et al.[9] was useful for studying tetanus immunity in a developing country and provides an alternative to the standard in vivo assay.

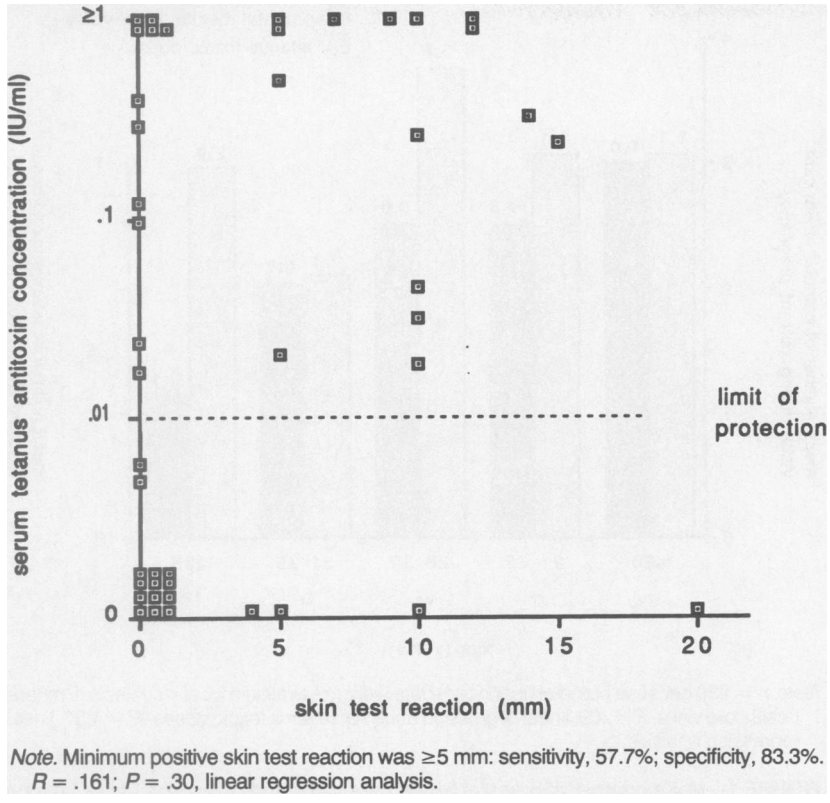
Roughly 40% of hospitalized pregnant Peruvian women lack sufficient serum tetanus antitoxin to protect their newborns against neonatal tetanus. That the rate in a jungle hospital was comparable to that of two Lima hospitals probably reflects recent vaccination efforts to target the jungle region of Peru, an area traditionally endemic for tetanus.

The overall 60% rate of protection exceeds the 1990 World Health Organization estimate of 29% for pregnant women in the developing world.[1] Our survey is, however, limited to hospitalized women. We would expect the coverage among women giving birth outside of hospitals to be lower owing to decreased access to health care.

A striking finding of our study was that a large percentage of women receiving no tetanus toxoid injections during their pregnancy reported one and often more prenatal health care visits. Clearly, many opportunities for vaccination were missed in our study population.

Our results confirm the highly significant correlation between the number of tetanus toxoid doses during pregnancy and the protective antitoxin titers. Among those subjects reporting two or three vaccinations during the pregnancy, almost 90% had protective antibody titers. Yet 40% of the subjects reporting no vaccinations during the pregnancy nevertheless had protective titers. Their immunity may represent either previous vaccination or naturally acquired antibody. Data from India and Brazil indicate that 3% to 40% of nonvaccinated subjects have positive antitoxin titers.[12–14] These findings suggest that health care workers can accept a patient's report of recent vaccinations, although a report of no recent vaccinations does not rule out immunity.

Younger women in our study were vaccinated more frequently than older women. Because we found no relation-



Note. Minimum positive skin test reaction was ≥5 mm: sensitivity, 57.7%; specificity, 83.3%. $R = .161$; $P = .30$, linear regression analysis.

FIGURE 2—Serum tetanus antitoxin level as determined by the modified ELISA vs the maximum diameter of induration with the tetanus toxoid skin test.

ship between age and the number of prenatal health care visits, the younger women either were offered vaccination more frequently or accepted it more often.

The tetanus toxoid skin test was of little value as a predictor of tetanus immunity. Factors responsible for the many false-negative results may include a decrease in cellular immune response to specific antigens in pregnant women near term[15,16] and the time period between vaccination and skin testing.

Neonatal tetanus remains an important cause of neonatal mortality in the developing world. Even in a population of Peruvian women with access to health care, tetanus vaccination rates remain far from ideal. Further studies to determine the reasons for missed vaccination opportunities would be helpful.

The modified ELISA was useful for investigating tetanus immunity in a developing country. Its wider application would facilitate study of other Third World populations. □

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