Tetanus and diphtheria antibodies and response to a booster dose in Brazilian HIV-1-infected women

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Received 4 July 2003; accepted 9 March 2004

Available online 9 April 2004

Abstract

Tetanus and diphtheria (Td) antibodies were studied in HIV-1-infected women during puerperium. HIV group (n = 61) was compared with Control group (n = 101). Twenty-one women from HIV and 13 from Control group who had antibody levels lower than 0.1 IU/mL received a booster with Td vaccine. Antibodies were assessed by double antigen ELISA. Mean tetanus and diphtheria antibody levels from HIV group were lower than those from Control group. Multiple linear regression analysis showed that tetanus and diphtheria antibody levels were decreased by HIV-1-infection, and that was independent of the reduction due to the time interval between last booster and antibody assessment. After a booster dose, both groups had an increase in mean tetanus and diphtheria antibody levels, but in Control group the levels were higher than in HIV group.

Keywords: Tetanus; Diphtheria; HIV infection

1. Introduction

Tetanus and diphtheria (Td) are life-threatening diseases that can be prevented through immunization. After routine vaccination, booster doses are necessary to maintain antibody levels [1]. In areas where diphtheria is still endemic, natural boosters can occur and that will contribute to increase diphtheria antibodies [2].

Tetanus toxoid administration is especially relevant in women of childbearing age to prevent neonatal tetanus, so that during pregnancy, the time interval between boosters should be shortened to 5 years [3]. As for diphtheria, a clear example of the risk of enlarging pool of susceptible persons was the diphtheria outbreak in countries belonging to the ex-URSS in the nineties [4].

In HIV-seropositive individuals who were infected after primary immunization, antibody levels against vaccine preventable diseases tend to be similar to those found in general population [5]. However, especially for antigens that need regular boosters such as tetanus and diphtheria, antibody decay tends to be faster in HIV-infected subjects [6].

Previous studies have already assessed tetanus and diphtheria antibodies in HIV-infected individuals [7–12]. However, small numbers of patients were tested and, except for one, they have not focussed on women of childbearing age. Considering the potential risk of low antibody levels for both women and their offspring in developing countries, we have evaluated tetanus and diphtheria antibodies in that population, and have compared them with healthy uninfected controls.

2. Material and methods

2.1. Study population

The present study was conducted from January 1998–2002 at the Federal University of São Paulo, in São Paulo, Brazil. This work was submitted to Ethics Committee of the Federal University of São Paulo, Brazil and written consent was obtained from all patients. Participants were women in the puerperium who were divided in two groups: HIV group, with HIV-1-infected...
women whose children were regularly followed up at the Pediatric AIDS Clinic. Control group, with healthy HIV-seronegative women who had given birth at the Maternidade Hospital of the Federal University of São Paulo.

HIV-1 infection was defined using the CDC criteria [13]. Women from HIV group who had recent available CD4+ T lymphocyte count ≥500, <500 and ≥200 and <200 cells/μL.

For each participant a form was completed with information about age, previous immunization, antenatal care and most recent CD4+ T lymphocyte count. Women who referred at least one dose of tetanus and diphtheria vaccine in the last 10 years before this study were selected for antibody analysis.

2.2. Blood sample collection

Venous blood samples were collected from all participants at study entry. For individuals who received a booster dose of Td, blood was also drawn at two other occasions: up to 90 days before vaccination and 25–90 days after.

Samples were centrifuged at 800 × g for 10 min. Serum was separated and stored at −70 °C until tested.

2.3. Immunization

Thirteen women in Control group and 21 in HIV group with tetanus and/or diphtheria antibodies lower than 0.1 IU/mL received a booster dose with Td vaccine (Butantan Institute, Brazil). This vaccine contained 10 Lf/dose of tetanus toxoid and 2 Lf/dose of diphtheria toxoid.

2.4. Measurement of antibodies

Tetanus and diphtheria antibodies were measured by double antigen ELISA as described by Kristiansen et al. [14]. For tetanus antibodies, 0.08 μg/ml of tetanus toxoid (Butantan Institute, Brazil) diluted in 0.1 M carbonate-bicarbonate buffer, pH 9.6, were used to coat 96-well microtiter plates (Dynex, USA) overnight at 4 °C.

Two-fold serial dilutions of serum samples and of tetanus reference serum (in house standard calibrated against “tetanus antitoxin human immunoglobulin NIBSC reagent 1976 (76/589)”) in dilution buffer (0.01M PBS, pH 7.2, 1% Triton X-100) with 1% bovine serum albumin (BSA) were added to the plate and incubated for 1 h at 37 °C. In the next step, biotin-labeled tetanus toxoid in dilution buffer was added to the plate and incubated for 1 h at 37 °C. Streptavidin-alkaline phosphatase conjugate (Zymed, USA) in dilution buffer was incubated for 1 h at 37 °C. p-Nitrophenyl-phosphate (Sigma, USA) in 1 M diethanolamine, 0.005 M magnesium chloride buffer, pH 9.8, was used as substrate and the absorbance was read at 450 nm in an immunorader ELX-800 (Bio-Tek Instruments, USA). Between steps, the plate was washed five times in dilution buffer.

For diphtheria antibodies, the same method was applied with some modifications: 0.05 μg/ml of diphtheria toxoid (Butantan Institute, Brazil), diphtheria reference serum (in house standard calibrated against “diphtheria antitoxin human serum 91/534”—NIBSC reagent) and biotin-labeled diphtheria toxoid were used as substitutes for the correspondent tetanus reagents.

Tetanus and diphtheria antibodies were expressed in International Units per millilitre (IU/mL) using the curve comparison method to transform optical density in concentration units.

2.5. Protective antibody levels

Patients were classified according to internationally accepted criteria of protective antibody levels [15-19]. Tetanus and diphtheria antibody concentrations lower than 0.01 IU/mL were considered without protection; levels between 0.01 and 0.09 IU/mL, basic immunity; and levels greater than 0.1 IU/mL, full protection.

2.6. Statistical analysis

For statistical analysis, sera without detectable antibodies were arbitrarily assigned the value of 0.005 IU/mL. Geometric means of tetanus and diphtheria antibodies were calculated from raw data. Due to non-normal distribution of values, they were submitted to logarithmic transformation before analysis.

Mean antibody levels were compared using Student’s t-test and ANOVA, with differences among groups assessed by Tukey comparison. Fisher exact and chi-square tests were used to compare proportions of individuals in each group in relation to age, origin, antenatal care and time interval between last booster and antibody assessment.

Multiple linear regression analysis was used to investigate the interference of each studied variable in tetanus and diphtheria antibody levels.

3. Results

3.1. Characteristics of study population

All participants were women in puerperium. The Control group consisted of 101 women and the HIV group, 61 women. The mean age in Control group was 27 years (range, 16–43 years) and in HIV group was 30 years (range, 19–44) (Student’s t-test, P = 0.002). The two groups did not differ in terms of antenatal care during the pregnancy (Control group: 97.5%; HIV group: 96.7%; Fisher exact test, P = 0.063), origin (women born in the State of São Paulo—Control group: 54.9%; HIV group: 55.0%; P = 0.995) and the time interval between last booster and antibody assessment Control group: 1.5 years (range, 0.01–8.0);
Table 1: Epidemiologic characteristics of the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>HIV</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, in years (range)</td>
<td>27 (16–43)</td>
<td>30 (19–44)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Antenatal care during pregnancy</td>
<td>97.5%</td>
<td>96.7%</td>
<td>0.063*</td>
</tr>
<tr>
<td>Women born in the State of São Paulo</td>
<td>54.9%</td>
<td>55.0%</td>
<td>0.995</td>
</tr>
<tr>
<td>Mean time interval, in years (range)</td>
<td>1.5 (0.01–8.0)</td>
<td>0.7 (0.1–8.0)</td>
<td>0.617*</td>
</tr>
</tbody>
</table>

* Student’s t-test.
* Fisher exact test.
* Chi-square test.

HIV group: 0.7 years (range, 0.1–8.0; Student’s t-test, P = 0.617). The epidemiologic characteristics of the individuals enrolled in the study are summarized in Table 1.

3.2. Seroprevalence of tetanus and diphtheria antibodies

Mean tetanus antibody levels were significantly lower in HIV group (0.22 IU/mL) than in Control group (1.34 IU/mL), (Student’s t-test, P < 0.001) (Fig. 1A). The same was observed for diphtheria antibodies, with mean levels in HIV group (0.41 IU/mL) significantly lower than those from Control group (1.83 IU/mL) (Student’s t-test, P = 0.001) (Fig. 1B).

The percentage of individuals with full protection against tetanus in HIV group (71%) was lower than in Control group (91%) (Student’s t-test, P < 0.001) (Fig. 1A). For diphtheria, the percentage of individuals with antibody levels ≥0.1 IU/mL was also lower in HIV group (76%) than in Control group (94%) (Student’s t-test, P = 0.001) (Table 2). No statistical difference was noticed between HIV and Control groups when women were divided using 0.01 IU/mL antibody levels for diphtheria (Fisher exact test, P = 0.557). However, for tetanus, the proportion of women in Control group with antibodies lower than 0.01 IU/mL (2%) was smaller than that of women in HIV group (11%) (Fisher exact test, P = 0.027).

Table 2: Distribution of women from Control and HIV groups in categories of immunity for tetanus and diphtheria

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus* (IU/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.01</td>
<td>2% (2/101)</td>
<td>11% (7/61)</td>
</tr>
<tr>
<td>≥0.01 and &lt;0.1</td>
<td>7% (7/101)</td>
<td>18% (11/61)</td>
</tr>
<tr>
<td>≥0.1</td>
<td>91% (92/101)</td>
<td>71% (74/101)</td>
</tr>
<tr>
<td>Diphtheria* (IU/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.01</td>
<td>1% (1/101)</td>
<td>3% (2/61)</td>
</tr>
<tr>
<td>≥0.01 and &lt;0.1</td>
<td>5% (5/101)</td>
<td>21% (13/61)</td>
</tr>
<tr>
<td>≥0.1</td>
<td>94% (95/101)</td>
<td>76% (46/61)</td>
</tr>
</tbody>
</table>

* The proportion of women in Control group with tetanus antibody levels ≥0.1 IU/mL (91%) was higher than that of women in HIV group (71%) (χ²: P = 0.001). The proportion of women in Control group with tetanus antibody levels <0.01 IU/mL (2%) was smaller than that of women in HIV group (11%) (Fisher exact test, P = 0.027). The proportion of women in Control group with diphtheria antibody levels ≥0.1 IU/mL (96%) was higher than that of women in HIV group (76%) (χ²: P = 0.001). The proportion of women in Control group with diphtheria antibody levels <0.01 IU/mL (1%) was similar to that of women in HIV group (3%) (Fisher exact test, P = 0.557). However, for tetanus, the proportion of women in Control group with antibodies lower than 0.01 IU/mL (2%) was smaller than that of women in HIV group (11%) (Fisher exact test, P = 0.027).

3.3. Tetanus and diphtheria antibodies according to CD4+ T lymphocyte counts

Mean tetanus antibody levels in HIV-1-infected individuals subdivided according to CD4+ T lymphocyte counts were significantly lower in two subgroups (CD4+ ≥500 and <500 and ≥200) when compared with Control group (Tukey comparison, P = 0.01 and 0.01, respectively) (Fig. 2A). For diphtheria antibodies, the same situation was observed: HIV subgroups CD4+ ≥500 and CD4+ < 500 and ≥200.
showed a statistical difference from Control group (Tukey comparison, \( P = 0.01 \) and 0.03, respectively) (Fig. 2B). No differences were found among HIV subgroups neither for tetanus nor for diphtheria antibodies.

### 3.4. Multiple linear regression analysis

Multiple regression analysis showed that HIV infection per se reduced tetanus antibody levels in 1.71 IU/mL (\( P < 0.001 \)); for diphtheria, the reduction was in 1.47 IU/mL (\( P = 0.011 \)). On the other hand, independently of HIV status, antibody levels proved to be inversely correlated with the time since last immunization, decreasing them by 0.22 IU/mL, for tetanus (\( P = 0.004 \)) and by 0.21 IU/mL, for diphtheria (\( P = 0.005 \)) for each year. Mean tetanus antibodies were also decreased by 0.06 IU/mL, for each year of age (\( P = 0.008 \)).

To be born in a Brazilian State other than the State of São Paulo decreased tetanus antibody levels by 0.62 IU/mL (\( P = 0.040 \)), and increased diphtheria antibodies by 0.73 IU/mL (\( P = 0.011 \)) (Table 3).

### 3.5. Tetanus and diphtheria antibodies after one Td dose

We compared the response to one Td vaccine dose in 21 women from HIV and 13 women from Control group. Before booster, women from both groups had tetanus and/or diphtheria antibodies lower than 0.1 IU/mL. Mean antibody levels did not differ either 0.04 and 0.04 IU/mL for tetanus (Student’s t-test, \( P = 0.43 \)) and 0.02 and 0.04 IU/mL for diphtheria (Student’s t-test, \( P = 0.085 \)) in HIV and Control groups, respectively.

After a booster dose, both groups had an increase in mean tetanus and diphtheria antibody levels. Mean tetanus post-booster antibody levels were significantly lower in HIV group (2.94 IU/mL) than in Control group (7.87 IU/mL) (Student’s t-test, \( P = 0.025 \)). HIV group had also lower mean diphtheria antibody levels after booster (0.55 IU/mL) than Control group (2.94 IU/mL), with \( P \) value close to significance level (Student’s t-test, \( P = 0.069 \)) (Fig. 3).

Due to the small number of women who were assessed for the response to a booster Td dose, we were not able to subdivide the HIV group according to CD4+ T lymphocyte levels.

### 4. Discussion

In this study, we have tested tetanus and diphtheria antibodies in Brazilian HIV-1 infected women of childbearing age. We chose this population because, apart from assessing specific immunity to these diseases in the women, we would
be indirectly evaluating their capacity to transmit tetanus passive immunity to their offspring in the event of a future pregnancy. Women from HIV and Control groups had similar epidemiologic characteristics, except for age (Table 1). As tetanus and diphtheria antibodies tend to decrease with time [16,20], we were aware that difference in age between groups might introduce a bias in the study. However, results from multiple regression analysis proved that other variables were independently associated with antibody levels.

Both tetanus and diphtheria antibodies were lower in HIV group when compared with Control group (tetanus, 1.34 IU/mL versus 0.22 IU/mL; diphtheria, 1.83 IU/mL versus 0.41 IU/mL) (Fig. 1A and B). Previous studies have not found differences in tetanus [11] or diphtheria antibodies [10] when HIV and healthy controls were compared. This lack of difference might be due to different laboratory techniques to assess antibodies with different degrees of accuracy [11]. Also, some researchers had small sample sizes [10], what might have interfered with their results. On the other hand, as will be discussed later, studying a group of HIV-infected individuals with a less altered immune system might lead to a reduced difference with healthy subjects.

The distribution of both groups in categories for tetanus and diphtheria (Table 2) also showed a lower proportion of HIV-infected women fully immune to tetanus and diphtheria (≥0.1 IU/mL). We have also observed a higher proportion of women from HIV group who were susceptible to tetanus (<0.01 IU/mL). The large percentage of HIV-infected women in the intermediate immune category for both diseases probably reflects the effect of a faster antibody decay and a lower response to booster doses in that population [6].

We expected to find a progressive reduction in mean antibody levels when women from HIV group were divided into immunologic categories for HIV infection. However, no difference among HIV subgroups was observed. Although this finding may indicate a qualitative derangement in early phases of HIV disease, it may also reflect a small sample size in different subgroups.

Kroon et al. [10] did not find statistically lower diphtheria antibodies in HIV-infected individuals, and found lower tetanus antibodies only in those with CD4+ T cells lower than 100 cells/μL when compared with Control HIV-negative subjects.

We have then performed multiple regression analyses to assess the effect of each separate variable on tetanus and diphtheria antibodies. We have shown that HIV infection was associated with a strong reduction in both tetanus and diphtheria mean antibodies and that was independent of the effect of time interval between last booster and antibody assessment, which was also associated with lower antibodies. Interestingly, older age was associated with a mean decrease in tetanus but not diphtheria antibodies. Other authors have found that susceptibility to both diseases increased with age [18,21–24].

Higher mean diphtheria antibodies were associated with women who were born in Brazilian States other than the State of São Paulo. While diphtheria is controlled in the latter, cases of the disease still occur in other Brazilian regions [25]. We then hypothesize that more frequent natural boosters might have contributed to the association observed through multiple regression analysis.

Lastly, we assessed the effect of a booster Td dose in women from both groups who had shown low tetanus and/or diphtheria antibodies (lower than 0.1 IU/mL). They were individuals who really needed the intervention and pre-booster antibody assessment was similar in both groups. Our results have shown that women from HIV and Control groups are
able to respond to the vaccination. However, the magnitude of the response is larger for Control group, whose individuals have reached higher antibody levels for both tetanus and diphtheria (Fig. 3 A and B).

Other groups have already shown similar results to a Td dose [10,12]. That reinforces the necessity of keeping HIV-infected individuals up-to-date with their vaccination. Moreover, women of childbearing age, apart from being protected, can also transfer tetanus antibodies to their offspring in the event of future pregnancy. And because placental transfer is reduced in that population [11], antibody levels must be higher than those found in a non-HIV-infected population.

Finally, the presence of a pool of susceptible individuals in any population can contribute to the triggering of a diphtheria epidemic. That has already been proved in a recent past [4] and must be avoided.

Acknowledgements

We thank the staff from the Department of Preventive Medicine, Federal University of São Paulo, Brazil, for assistance in statistical analyses. We thank Dr. Abês Mahmed Amed, Department of Obstetrics of the Federal University of São Paulo, Brazil, for assistance in data collection. We thank Dr. Reinaldo Salomão, Division of Infectious Diseases of the Federal University of São Paulo, Brazil, for reviewing the manuscript. This work was supported by FAPESP, Brazil—protocol number: 00/01332-7 and 97/06118-9.

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