Associations of toluidine red unheated serum test response to the treatment of syphilis in pregnancy and congenital syphilis: a 10-year real-world study

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Abstract

Background: So far, there is a paucity of real-world data on the syphilis serological responses to the first-line treatment during pregnancy, and there is no relevant study on the necessity of anti-syphilis treatment during pregnancy for those patients who have been treated for syphilis before pregnancy for the prevention of mother-to-child transmission, which might provide valuable insight into treatment effectiveness and optimal management of pregnant women with syphilis.

Methods: A retrospective study on 10 years of real-world data was performed for accumulative 410 Chinese pregnant women with syphilis. The descriptive statistics were conducted in the study, and toluidine red unheated serum test (TRUST) titer responses to penicillin treatment in syphilis-infected pregnant women, and the associations with congenital syphilis were investigated. We divided the patients into two groups according to the history of anti-syphilis treatment before pregnancy (patients diagnosed with syphilis who had received anti-syphilis treatment before pregnancy, and patients screened and diagnosed with syphilis during pregnancy who had no previous history of anti-syphilis treatment).

Results: The rate of congenital syphilis in this study was 6.2% (25/406). There was no significant difference in the rate of congenital syphilis between patients who received anti-syphilis treatment before pregnancy and those who did not. Secondary syphilis and high baseline serum TRUST titer ($\geq$1:8) in pregnant women were independent risk factors for congenital syphilis.

Conclusions: For the prevention of congenital syphilis, anti-syphilis treatment during pregnancy for syphilis seropositive pregnant women is needed, regardless of whether the patient has received anti-syphilis treatment before pregnancy, especially for those patients with secondary syphilis or high baseline serum TRUST titer, thus, timely surveillance, early diagnosis to timely treatment, and close syphilis reexamination during posttreatment follow-up, may help to reduce the above-mentioned risk factors for congenital syphilis.

Keywords: Syphilis; Pregnant syphilis; Toluidine red unheated serum test (TRUST)

Introduction

Syphilis, caused by *Treponema pallidum* (*T. pallidum*),[1] is a chronic, infectious human disease, and remains the most common congenital infection worldwide.[2] Syphilis in pregnancy is under global public health concern[3] because this pathogen can be transmitted vertically to the fetus and can cause tremendous consequences for the mother and her developing fetus, including abortion, stillbirth, and congenital syphilis.[4-7] In 2007, the World Health Organization (WHO) launched an initiative to eliminate congenital syphilis by 2015 with goals to test 95% of gravidas for syphilis and treat 95% of seropositive gravidas.[8] And in response to this initiative established by

WHO, several recent studies have examined the current status of syphilis prevention and control in developing countries.[9] For example, Wan *et al*[10] have investigated the impact of maternal treatment during pregnancy on pregnancy outcomes in Jiangxi province, China, by comparing pregnancy outcomes with respect to different treatment statuses. Hu *et al*[11] conducted a retrospective cohort study to investigate the effect of different treatment regimens and multiple risk factors on adverse pregnancy outcomes among syphilis-seropositive women in Guangzhou, China. So far, penicillin remains the first choice to treat syphilis in pregnancy and prevent congenital syphilis. However, there is a paucity of real-world data on the syphilis serological responses to the first-line anti-syphilis

treatment during pregnancy, and the possible influencing factors for the above. Additionally, there are no relevant studies on the necessity of anti-syphilis treatment during pregnancy for the prevention of mother-to-child transmission (MTCT) for those patients who received anti-syphilis treatment before pregnancy. The present study aimed to address this research gap. And the findings may provide valuable insight into treatment effectiveness and optimal management of pregnant women with syphilis.

Methods

Ethical approval

The study was approved by the Institutional Review Board of Beijing Ditan Hospital, Capital Medical University (No. JDLKZ2018-033-02), and conducted following ethical standards. Each patient provided written informed consent.

Selection and description of participants

We collected the real-world data of all pregnant women with syphilis presenting to the Department of Dermatology and Venereology (a branch of the National Clinical Key Department of Infectious Diseases), Beijing Ditan Hospital, Capital Medical University, Beijing, from January 2008 to December 2017 through medical records.

Syphilis was diagnosed based on syphilis serological results, clinical evidence, and previous medical history. The traditional screening algorithm for syphilis begins with a non-treponemal test, such as the rapid plasma reagin test, toluidine red unheated serum test (TRUST), or Venereal Disease Research Laboratory test. Then, reactive samples are confirmed by using one of the several treponemal tests. This algorithm performs well in identifying patients with active syphilis while reducing the false-positive rate in the low-prevalence population.[12] Patients with both TRUST and *Treponema pallidum* particle agglutination (TPPA) positive were diagnosed as syphilis-seropositive in this study.

Participants in the study were included as follows [Supplementary Figure 1, http://links.lww.com/CM9/B18]. All participants were pregnant women with syphilis before or during pregnancy, who were admitted to the above-mentioned hospital for treatment and delivery. We divided the patients into two groups according to the history of anti-syphilis treatment before pregnancy: one group comprised patients diagnosed with syphilis who had received anti-syphilis treatment before pregnancy, and the other group comprised patients screened and diagnosed with syphilis during pregnancy who had no previous history of anti-syphilis treatment.

Technical information

Since January 2008, all syphilis seropositive pregnant women presenting at the above-mentioned hospital have received treatment to prevent syphilis MTCT. The first-line standardized treatment in China before 2020 was two complete courses of anti-syphilis treatment with benza-thine penicillin G (BPG) intramuscularly in the first and the last trimester of pregnancy, respectively. In this study, 81.0% (332 cases) of patients received this standardized treatment; those patients who did not (78 cases, 19.0%) were late for their first hospital appointment and therefore missed the first course of treatment. In the first trimester of pregnancy, the gestational age is 12 weeks, whereas in the last trimester of pregnancy, the gestational age is 28 weeks. A single complete course includes BPG 2.4 million units intramuscularly per week for three consecutive weeks.

TRUST is used as a type of effective screening strategy in detecting the severity of syphilis[13] and as an auxiliary surrogate indicator for the diagnosis of congenital syphilis in China.[14] Serum TRUST titer of the patients before the initial treatment and in the month before delivery, as well as of their newborn babies at birth, were tested routinely and recorded. The diagnosis of congenital syphilis in newborns requires both TPPA and *T. pallidum* immuno-globulin M test result positive. In the absence of these results, the diagnosis of congenital syphilis can be made if newborns maintain reactive TRUST titers that are four times higher than their mothers' before delivery. Serum baseline TRUST titer refers to the patients' serum TRUST titer tested just before they receive anti-syphilis treatment to prevent MTCT.

Statistics

Statistical analysis was performed using the Statistical Package for Social Sciences version 19.0 (Chicago, IL, USA). Normally distributed measurement data were expressed as mean ± standard deviation. The between-group comparison of count data was performed using the chi-squared test. Non-normally distributed measurement data were expressed as Q2 (Q1, Q3), and the between-group comparison was performed using a non-parametric rank-sum test (Mann-Whitney U test). Categorical variables were presented as numbers and frequencies. Count data were described using frequency (percentage). The estimated risk of congenital syphilis was confirmed using logistic regression with 95% confidence intervals (CIs). Throughout the analysis, $P < 0.05$ was considered significant as a condition for rejecting the null hypothesis.

Results

A total of 410 pregnant syphilis patients were included in the study. The mean age was 30.0 ± 4.9 years (range: 18.0–46.0 years). Of the 410 pregnant syphilis patients, 406 had live births and four had stillbirths. Of the 406 live births, 25 (6.2%) had congenital syphilis

Serum TRUST titer response under treatment

We categorized patients according to their baseline serum TRUST titer into ≤1:8 and >1:8 groups, based on our clinical experience in China, and performed a chi-square analysis on data for the two groups. Patients' antenatal serum TRUST titer changes were significantly different ($P < 0.05$) between the two different baseline titer groups.

Table 1: Antenatal serum TRUST titer response under different baseline titers.

Antenatal TRUST titer response compared to baseline	Baseline titer ≤1:8, n (%)	Baseline titer >1:8, n (%)	χ^2	P values
Converted negative			20.865	<0.001[*]
Yes	54 (17.6)	0 (0)		
No	253 (82.4)	103 (100)		
Declined			65.641	<0.001[*]
Yes	109 (35.5)	84 (81.6)		
No	198 (64.5)	19 (18.5)		
Unchanged			14.109	<0.001[*]
Yes	108 (35.2)	16 (15.0)		
No	199 (64.8)	87 (84.5)		
Elevated			6.691	0.008[*]
Yes	36 (11.7)	3 (2.9)		
No	271 (88.3)	100 (97.1)		
Sum	307 (100)	103		

The corresponding definition of the above is as follows: (1) Converted negative: After treatment, the patient's TRUST test result became negative. (2) Declined: After treatment, the patient's TRUST titer decreased, but did not become negative. (3) Unchanged: After treatment, the patient's TRUST test result showed no change. (4) Elevated: After treatment, the patient's TRUST titer increased. [*]$P < 0.05$.

Table 2: Antenatal serum TRUST titer response under the different times of initial treatment in pregnancy.

Antenatal TRUST titer changes compared to baseline	Initial treatment time			χ^2	P values
	≤12 W	13–24 W	≥25 W		
Converted negative (%)	14.5	13.0	11.5	0.385	0.825
Yes (n)	18	27	9		
No (n)	106	181	69		
Declined (%)	45.2	50.0	42.3	1.608	0.448
Yes (n)	56	104	33		
No (n)	68	104	45		
Unchanged (%)	30.6	27.4	37.2	2.583	0.275
Yes (n)	38	57	29		
No (n)	86	151	49		
Elevated (%)	9.7	9.6	9.0	0.033	0.984
Yes (n)	12	20	7		
No (n)	112	188	71		
Sum	124	208	78		

W: Gestational weeks.

Specifically, antenatal serum TRUST titer was more likely to be converted negative, unchanged, and elevated in the baseline TRUST titer ≤1:8 group than that in the baseline TRUST titer >1:8 group. We also found that the antenatal serum TRUST titer was more likely to decline in the baseline TRUST titer >1:8 group [Table 1].

The antenatal serum TRUST titer changes among different initial treatment time groups were compared and the chi-square analysis for each was performed. As shown in Table 2, the antenatal serum TRUST titer changes among each group showed no statistical difference (all $P > 0.05$). Therefore, the groups did not differ on rates of the patient's TRUST titer converted negative, declined, unchanged, and elevated, and antenatal changes in serum TRUST titer were not related to the initial treatment time in different pregnancy periods.

We divided the patients into two groups according to the history of anti-syphilis treatment before pregnancy. The rank-sum test of the unidirectional ordered classification data of the two groups showed a Z value of −3.530, and the difference was significant, indicating that the antenatal serum TRUST titer changes in the two groups were different. We used a chi-square test to analyze the specific trends in serum TRUST titer before delivery in the two groups of pregnant women. As shown in Table 3, patients with no history of syphilis treatment before pregnancy were more likely to show a decline in antenatal serum TRUST titer, but the difference in negative conversion rate was not significant.

A comparison of the TRUST titer before labor with the baseline TRUST titer showed that the group with a history of anti-syphilis treatment before pregnancy had the same

Table 3: Antenatal serum TRUST titer response under different treatment history before pregnancy.

Antenatal TRUST titer response compared with baseline	Anti-syphilis treatment before pregnancy		χ^2	P values
	Yes	No		
Converted negative (%)	16.9	11.1	2.804	0.094
Yes (n)	25	29		
No (n)	123	233		
Declined (%)	25.7	52.3	27.383	<0.001*
Yes (n)	38	137		
No (n)	110	125		
Unchanged (%)	36.5	26.7	4.278	0.039*
Yes (n)	54	70		
No (n)	94	192		
Elevated (%)	21.6	9.9	10.656	0.001*
Yes (n)	32	26		
No (n)	116	236		
Sum	148	262		

* $P < 0.05$.

median value, whereas the group not treated before pregnancy had a lower median value than the baseline, and showed effective to the anti-syphilis treatment during pregnancy. There was no significant difference in baseline TRUST titer between the two groups.

Findings from univariate analysis

The univariate analysis showed that two factors (ie, clinical stage and baseline serum TRUST titer) were related to the rate of congenital syphilis, and the differences were significant [Table 4].

Moreover, a chi-square test was used to compare the rate of congenital syphilis between the two patient groups (patients who had anti-syphilis treatment history before pregnancy and those who had not). The results showed a P value of 0.309, indicating no significant difference in the rate of congenital syphilis in babies born to the two groups of pregnant women.

Findings from multiple logistic regression analysis

Based on the univariate analysis findings that clinical stage and baseline serum TRUST titer were associated with the rate of congenital syphilis, a stepwise multivariate logistic regression was used to fit a logistic model. The two significant risk factors in the univariate analysis were used as independent variables. The presence or absence of congenital syphilis was the dependent variable. The values of the baseline serum TRUST titer variable were as follows: 0 was used for baseline serum TRUST titer 1:1, 1 for 1:2, 2 for 1:4, 3 for 1:8, 4 for 1:16, 5 for 1:32, 6 for 1:64, and 7 for titers ≥1:128. The values of the syphilis clinical-stage variable were as follows: 0 was used for recessive syphilis, and 1 and 2 for syphilis in the first and second stages, respectively. The results showed that the

two independent risk factors for congenital syphilis entered into the model, maternal baseline serum TRUST titer and clinical stage, had odds ratios (ORs) of 2.822 and 1.320, respectively.

Of the clinical-stage factors, secondary syphilis had an OR of 11.394 (95% CI = 1.809–71.751) and the difference was significant. This indicates that pregnant patients with secondary syphilis had an 11 times higher risk of delivering a baby with congenital syphilis than did those with latent syphilis or primary syphilis.

Of the baseline serum TRUST titer factors, pregnant women's baseline serum TRUST titers of 1:8, 1:16, 1:64, and ≥1:128 were correlated with a higher risk of delivering a baby with congenital syphilis, and the difference was significant. When the baseline TRUST titer was ≥1:8, the possibility of congenital syphilis increased as the titer increased. The OR of the patient group with baseline serum TRUST titer of 1:32 was 0 because there were no patients in this group [Table 5].

Discussion

Principal findings

This study provided evidence from 10-year real-world data on serum TRUST titer responses of syphilis-infected pregnant women receiving first-line treatment with benzathine penicillin in China from January 2008 to December 2017. Current research on syphilis serological response features under the first-line treatment during pregnancy is limited. Therefore, our findings present a broadened view of the syphilis serologic features in pregnancy under benzathine penicillin treatment, and the associations with congenital syphilis. New findings in our study are as follows.

There was a significant difference in antenatal serum TRUST titer changes between the different baseline TRUST titer groups. We found that prenatal serum TRUST titer was more likely to be converted negative, unchanged, and elevated in the baseline TRUST titer ≤1:8 group, which suggests, respectively, that some patients with lower TRUST titers can be more easily cured, some patients may show serofast, and that further research is needed to explain the present findings regarding elevated serum TRUST titer.

Patients with a history of anti-syphilis treatment before pregnancy had no advantage in the decline rate of antenatal serum TRUST titer than patients with no history of such treatment, and the same pattern was observed for congenital syphilis rate. We found that patients who received treatment before pregnancy but still delivered fetuses with congenital syphilis had very high TRUST titers at both baseline and after treatment. The possible causes of this phenomenon (eg, reinfection, relapse, or non-standard treatment before pregnancy) deserve further study. The above reveals that, on the one side, anti-syphilis treatment during pregnancy for syphilis seropositive pregnant women is necessary to prevent

Table 4: Univariate analysis of the rate of congenital syphilis in pregnant women with syphilis.

Variables	Non-congenital syphilis, n (%) (N = 381)	Congenital syphilis, n (%) (N = 25)	χ^2	P values
Age (years)			3.132	0.077
≤25	67 (17.6)	5 (20.0)		
26–30	145 (38.1)	3 (12.0)		
31–35	122 (32.0)	11 (44.0)		
>35	47 (12.3)	6 (24.0)		
Occupation			0.166	0.733
Fixed employment	116 (30.5)	7 (28.0)		
Farmer	13 (3.4)	0 (0)		
Unemployment	217 (57.0)	16 (64.0)		
Other vocation	35 (9.2)	2 (8.0)		
Status of residence			0.549	0.459
Beijing resident	150 (39.4)	8 (32.0)		
Non-Beijing resident	231 (60.6)	17 (68.0)		
Ethnic			1.074	0.300
Han	368 (96.6)	23 (92.0)		
Minority	13 (3.4)	2 (8.0)		
Education			0.260	0.610
Below high school	121 (31.8)	9 (36.0)		
High school	105 (27.6)	7 (28.0)		
College or above	155 (40.7)	9 (36.0)		
Adverse pregnancy history			3.434	0.614
No	125 (32.8)	4 (16.0)		
Yes	256 (67.2)	21 (84.0)		
Parity			0.416	0.519
Unipara	81 (21.3)	4 (16.0)		
Multipara	300 (78.7)	21 (84.0)		
Syphilis stage			6.638	0.010*
Latent syphilis	376 (98.7)	22 (88.0)		
Primary syphilis	2 (0.5)	1 (4.0)		
Secondary syphilis	3 (0.8)	2 (8.0)		
Initial treatment time			0.674	0.412
≤12 W (2 courses)	116 (30.5)	7 (28.0)		
13–24 W (2 courses)	194 (50.9)	11 (44.0)		
>25 W (1 course)	71 (18.6)	7 (28.0)		
Treatment before pregnancy			0.806	0.309
Yes	241 (63.3)	18 (72.0)		
No	140 (36.8)	7 (28.0)		
Serum TRUST baseline titer			11.398	0.001*
1:1	91 (23.9)	1 (4.0)		
1:2	97 (25.5)	3 (12.0)		
1:4	61 (16.0)	5 (20.0)		
1:8	42 (11.0)	6 (24.0)		
1:16	35 (9.2)	4 (16.0)		
1:32	29 (7.6)	0 (0)		
1:64	19 (5.0)	4 (16.0)		
≥1:128	7 (1.8)	2 (8.0)		

* $P < 0.05$. W: Gestational weeks.

syphilis MTCT, regardless of whether the patient had anti-syphilis treatment history before pregnancy, especially for those patients with secondary syphilis or high baseline serum TRUST titer, as it may help to reduce the above-mentioned risk factors for congenital syphilis. On the other side, timely syphilis surveillance, early diagnosis to enable timely treatment, standardized anti-syphilis treatment, and close syphilis reexamination during posttreatment follow-up are also of significance, as these measures may prevent syphilis recurrence, particularly in individuals with multiple syphilis episodes. This is an important finding as it suggests a clear public health message for patients with syphilis. And the latter part of the above message has also been proven by an investigation among HIV-infected syphilis patients.[15]

We found that secondary syphilis and higher baseline serum TRUST titer (≥1:8) in pregnant women were, respectively, independent risk factors for congenital syphilis.

Table 5: Multivariate logistic regression analysis: correlates of the rate of congenital syphilis.

Variables	P values	OR	95% CI
Syphilis infection stage	0.027*	2.822	(1.127–7.063)
Latent syphilis	0.009*	1.000	
Primary syphilis	0.085	8.545	(0.746–97.910)
Secondary syphilis	0.010*	11.394	(1.809–71.751)
Serum baseline TRUST titer	0.006*	1.320	(1.083–1.609)
1:1	0.065	1.000	
1:2	0.374	2.814	(0.288–27.548)
1:4	0.070	7.459	(0.85–65.419)
1:8	0.019*	13.000	(1.517–111.418)
1:16	0.039*	10.400	(1.123–96.305)
1:32	0.998	0	
1:64	0.010*	19.158	(2.207–181.108)
≥1:128	0.011*	26.000	(2.091–323.322)

$^{*}P < 0.05$. CI: Confidence interval; OR: Odds ratio.

Results of the study in the context of other observations

Several of the present findings are consistent with previous reports that one course of penicillin treatment during pregnancy results in similar adverse outcome rates as two courses of treatment,[16,17] and that the risk of congenital syphilis rate in pregnant women with secondary syphilis is higher than in those with latent syphilis.[18] In 2020, the Chinese guideline for the prevention of syphilis MTCT was changed to one course of anti-syphilis treatment during pregnancy for syphilis-infected pregnant women.[19]

In contrast to previous findings,[18] we found that patients with primary syphilis did not have a higher rate of congenital syphilis than those with latent syphilis. Of the 410 patients, 97.8% had latent syphilis. Primary syphilis is more easily diagnosed owing to its manifested symptom and can be treated promptly, resulting in the reduction of both the number and the risk of transmission in the primary syphilis group.

In the present study, the initial treatment time for syphilis during pregnancy had no significant relationship with antenatal serum TRUST titer changes compared with baseline, the same pattern was found for the rate of congenital syphilis, which is inconsistent with the previous findings.[20] Considering the disproportionate patient numbers and the unevenness of TRUST titer baselines across the three treatment groups in this study, further investigations with balanced participant numbers in each group are needed for confirmation.

Strengths and limitations

Some limitations should be noted in this study. First, the small number of patients in the first clinical stage of syphilis enrolled in this study due to the real data might have limited the power of the study to detect differences in risk. Second, because of a lack of clarity in some patient medical records, we did not differentiate outcomes according to when patients were first diagnosed with syphilis and at which time point they were treated before

pregnancy. Therefore, future studies are needed that take this information into account.

However, our study advances the literature by conducting a more in-depth examination of pregnant patients' serum TRUST titer responses, and we provide more details about several aspects of serological response to first-line therapy for pregnant women with syphilis. We successfully analyzed the potential influencing factors for the rate of congenital syphilis, which may be useful to optimize the treatment of syphilis during pregnancy.

An additional strength of our study was that this was a 10-year real-world retrospective study. These data provide much-needed evidence to help obstetricians and dermatologists make important clinical decisions about the optimal approach for the management of patients with syphilis in pregnancy. The factors examined here are carefully evaluated in clinical decision-making for women with syphilis in pregnancy but are seldom considered in larger database-driven studies.

Conclusions and clinical implications

To our knowledge, this is the first study to investigate, evaluate, and confirm the efficacy of first-line treatment with benzathine penicillin for syphilis in pregnancy using serum TRUST titer responses. The findings may help to promote, at least partly, the optimal management of syphilis in pregnancy. The present findings have the following clinical implications.

First, anti-syphilis treatment during pregnancy is the key to improving pregnancy outcomes among serosyphilis positive women, and cannot be substituted by anti-syphilis treatment before pregnancy, which clarify and support the recent consensus guidelines on the prevention of MTCT in syphilis.[2,21] Second, since high baseline serum TRUST titers were positively associated with the rate of congenital syphilis, a delay in initiating treatment for pregnant women with high baseline serum TRUST titers may increase the exposure risk of the fetus to *T. pallidum*. Thus, it is necessary to strengthen follow-up and reexamination for pregnant women with syphilis, and whether another course of anti-syphilis treatment is needed depends mainly on the serosyphilis titer results, to reduce the amount and activity of syphilis pathogens in patients as soon as possible, and improve pregnancy outcomes.

Conflicts of interest

None.

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