Efficacy of doxycycline in the treatment of syphilis

Running title: Efficacy of doxycycline in syphilis

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ABSTRACT

Doxycycline is an alternative antibiotic drug in treating syphilis, but the efficacy data, especially the data on late latent syphilis, are limited. A retrospective study was conducted to evaluate the effectiveness of doxycycline in treating patients with different stages of syphilis. Patients who received doxycycline treatment between June 2011 and June 2014 were involved. Serological response to doxycycline was defined as either negative TRUST test result or a ≥4-fold decrease in titers at 12 months following the treatment. Univariate and multivariate logistic regression analyses were performed to identify factors associated with serological response. During the study period, a total of 163 syphilis patients were treated with doxycyclinea, and 118 patients completed doxycycline treatment and 12-month follow-up. Among the 118 patients, the serological response rate at 12 months was 100.0% (7/7) in primary syphilis, 96.9% (62/64) in secondary syphilis, 91.3% (21/23) in early latent syphilis, and 79.2 (19/24) in late latent syphilis. The total serological response rates were 92.4% (109/118) for pre-protocol (PP) patients and 66.9% (109/163) for all intention-to-treat (ITT) patients. In multivariate analysis, patients who serologically responded at 12 months following treatment were positively associated with a higher baseline TRUST titer and an earlier syphilis stage than nonresponders. Our study showed excellent treatment outcomes in different stages of syphilis patients. Our data, along with other reports, support the usage of doxycycline as a good alternative therapeutic option in the treatment of syphilis.
Key Words Syphilis, Doxycycline, Efficacy

INTRODUCTION

Syphilis is a serious infection that can cause acute cutaneous manifestations, chronic compromise of the cardiovascular and nervous systems, and serious effects on reproductive and neonatal health. Syphilis also increases the risk of HIV acquisition and transmission (1). The World Health Organization estimated 10 million new infections still occur each year (2). Syphilis remains a worldwide public health problem. Without a vaccine, efficient diagnosis and treatment of syphilis is essential for effective syphilis control.

Both the United States and European guidelines recommended parenterally administered penicillin G for the treatment of all stages of syphilis (3-4). Although benzathine penicillin is the recommended first-line treatment for syphilis in China, it is not available in many hospitals in China. Doxycycline, tetracycline and azithromycin were preferred alternate agents in patients allergy to penicillin, especially to those who not tolerate intramuscular injection. Early success of azithromycin had led to considerable enthusiasm (5-7). However, an increased number of azithromycin treatment failures associated with a 23S rRNA mutation for macrolide resistance were reported from several areas in recent years (8-11). Macrolide resistance is extremely high in China, with the 23S rRNA A2058G...
mutation presented in 91.9% of the patients from a national survey (12). The azithromycin treatment failure was reported in Shanghai (13) and our previous study shown Shanghai 23S rRNA A2058G mutation ranked the highest in China (14). Tetracycline can cause more gastrointestinal side effects and requires more frequent dosing compared with doxycycline (15). Therefore, doxycycline is endorsed as an alternative preferred therapy. Regimens of doxycycline 100 mg orally twice daily for 14 days for early syphilis and 28 days for late syphilis have been used for many years. However, studies on doxycycline for the treatment of syphilis are rare and their results are partly contradictory (16-21). Early case series reported very high response rate about doxycycline treatment, but most with the dose different from current recommendations (16-17). Recent reports with the recommend doxycycline dose showed a much lower response rate (18, 21). Most of these reports about doxycycline were small case series and none of them included late stage of syphilis.

Decreased use of macrolides could result in increased use of tetracyclines/doxycycline. The potential of develop additional tetracyclines/doxycycline resistance due to selective pressure should raise our concern. To provide more convincing data about doxycycline treatment, we conducted a retrospective study to evaluate the response rates of patients with different stages of syphilis treated with the recommended dose of doxycycline, factors associated with serological response were also assessed.
MATERIALS AND METHODS

First-line medicine benzathine penicillin was not available at Shanghai Xuhui Central Hospital during the study period. Doxycycline as an alternative therapy was introduced to syphilis patients. Included subjects were consecutive adults ≥ 18 years of age who were diagnosed and agreed to treatment with doxycycline for syphilis at the sexually transmitted disease (STD) clinics in Shanghai Xuhui Central hospital (Shanghai Clinical Center, Chinese Academy of Science) between Jun 2011 and Jun 2014. Information about demographic characteristics, symptoms, sexual orientation, stage of syphilis, HIV-1 status, treatment and serological results were collected using a standardized clinical form. Primary, secondary, early and late latent syphilis diagnoses were made by trained clinicians. Primary syphilis is characterized by an ulcer or chancre, and laboratory confirmation of *Treponema pallidum* by positivity of both the *Treponema pallidum particle assay* (TPPA) test and non-treponemal test *Toluidine red unheated serum test* (TRUST). Secondary syphilis is generally characterized by skin rash, mucocutaneous lesions, usually with lymphadenopathy, and laboratory test confirmation. Latent syphilis was defined as an asymptomatic case detected by reactive TRUST and TPPA test. Latent syphilis acquired within the preceding year is classified as early latent syphilis, other cases of unknown duration or more than 12 months is referred to late latent syphilis.

**Laboratory Test** Laboratory analyses, including serum TRUST and TPPA, were conducted in accordance with the instructions of the manufacturers. The TRUST
reagents were manufactured by Shanghai Rongsheng Biotech and TPPA were manufactured by Fujirebio INC. All serum samples used in the TRUST test were diluted to avoid prozone effects and false negative results. The titers of the serum samples that were reactive with TRUST were quantified using twofold serial dilutions until the endpoint was determined. Among eligible participants, human immunodeficiency virus (HIV) serologic status was determined through a dual enzyme-linked immunosorbent assay.

**Treatment and follow-up** Doxycycline therapy consisted of 100 mg orally twice daily 14 days for early syphilis and 28 days for late latent syphilis. As a routine in our STD clinic, after treatment, all the patients were asked to periodically review their clinical symptoms and serum TRUST titers every 3 months in the first year and every 6 months in the following years. Serological response criteria were defined as either negative TRUST test result or a ≥ 4-fold decrease in titers at 12 months following treatment. Seroreversion mean a positive TRUST test becoming negative after treatment. To be included in the study, at least 2 visits (an initial titer at the time of treatment and at least 1 follow-up titer 12 months after the date of treatment) were necessary for the observation of serological titer changes. On the other hand, the serological response is calculated considering all the intention-to-treat patients.

**Statistical Methods** Data were recorded using Microsoft Excel™ and validated through double entry. Univariate and multivariate logistic regression analyses were performed using Statistical Package for the Social Sciences (version 17.0, SPSS Inc.,
Chicago, IL, USA) to identify factors associated with serological response. Odds ratios (ORs) and their corresponding 95% confidence intervals (CI) were produced to present factors associated with serological response. Multiple regression analyses were performed to adjust ORs for potential confounders. Only variables that were significant in univariate analyses were included in multivariable logistic regression models for selecting the independent risk factors. The Ethical Committee of the Shanghai Xuhui Central Hospital (Shanghai Clinical Center, Chinese academy of science) waived the need for institutional review board approval because of the retrospective nature of this study.
RESULTS

Study Population From Jun 2011 to Jun 2014, a total of 163 syphilis patients were treated with doxycycline. Of the 163 intention-to-treat (ITT) patients, 129 completed 6 months follow up, 118 completed 12 months follow up and were included in the study. 45 patients lost to 12 months follow-up, including 19 who did not attend any follow-up visits and were excluded from the study (Figure 1). Of the 19 patients, 3 didn’t complete doxycycline treatment because of impaired liver function tests.

Among the 118 pre-protocol (PP) participants, the median age was 48.0 years (ranged between 20 and 69 years of age), 67 (56.8%) were male, 9 (5.9%) were homosexual/bisexual and 2 (1.7%) were HIV positive. More than half of the patients (64, 54.2%) had secondary syphilis, 7 (5.9%) had primary syphilis, 23 (19.5%) had early latent syphilis and 24 (20.3%) had late latent syphilis (Table 1). Serum TRUST titers ranged from 1:1 to 1:512 before treatment with median titer 1:32.

Treatment and follow-up Of the 118 syphilis cases with available treatment outcome data, 94 (79.7%) treated with doxycycline 100mg twice daily 14 days for early syphilis and 24 (20.3%) treated with doxycycline 100mg twice daily 28 days for late syphilis. Late latent syphilis group had a lower median basic serum TRUST titer (Median TRUST titer 1:16 vs 1:64). The two treatment groups were generally similar with regard to socio-demographic and behavioral characteristics.

Serological response rate All the clinical symptoms of primary and secondary syphilis resolved after treatment. The total serological response rates were 92.4%
(109/118) for the 118 PP patients. The distribution of serological response according

to the clinical stage of the syphilis is described in Figure 2. At 12 months, the

serological response rate was 100.0% (7/7) in primary syphilis, 96.9%(62/64) in

secondary syphilis, 91.3(21/23) in early latent syphilis and 79.2(19/24) in late latent

syphilis respectively. Among these patients, seroreversion rate was 85.7%(6/7) in

primary syphilis, 39.1%(25/64) in secondary syphilis, 47.8%(11/23) in early latent

syphilis and 25%(6/24) in late latent syphilis. Median TRUST titer decrease was 16

folder at 12 month. The total serological response rates were 66.9% (109/163) for all

intention-to-treat (ITT) patients.

Among the 9 patients with serological failure, 1 patient with late latent syphilis had a

8-fold increase in titers (from 1:4 to 1:32) after therapy, 1 patient with secondary

syphilis relapsed at 9 months, the other 7 patients (1 secondary syphilis, 2 early latent

syphilis and 4 late latent syphilis) were patients who did not reach a 4-fold decrease in

their titers 12 months after therapy. Both the 2 HIV positive patients achieved

serological response.

Factors associated with serological response

In univariate analysis, patients who serologically-responded at 12 months following

treatment were positively associated with a higher baseline TRUST titer and an earlier

syphilis stage than non-responders. Multivariate logistic regression analyses also

showed that factors positively associated with serological response were: baseline

TRUST titer (>32, AOR 18.82, 95% CI (2.14-165.90), syphilis stage (early AOR 5.14,
95% CI (1.04-25.37), (Table 2).
DISCUSSION

The evidence upon which recommendations for syphilis therapy are based remains inadequate. The major advantages of Benzathine penicillin treatment are its safety, effectiveness, and the favorable adherence to the weekly dosing schedule. The most limiting factor to using Benzathine penicillin in China is its availability, though penicillin allergy remains an issue throughout the World. Doxycycline is a tetracycline derivative with better oral bioavailability, convenient twice a day dosing, and fewer gastrointestinal side effects (15, 22). As an alternative treatment, doxycycline presents several advantages.

Our study showed excellent treatment outcomes in early syphilis patients (response rate was 100.0% in primary syphilis, 96.9 % in secondary syphilis, 91.3% in early latent syphilis). The findings are consistent with early retrospective reports from Onoda, Harshan and Ghanem(16-18). Both Harshan and Ghanem reported 100% response rate in early syphilis. Similarly, 14 of 15 (93.3%) early syphilis patients had serological evidence of response to treatment in Onoda’s study. It should be pointed out that Onoda treated with repeated doxycycline therapy with double dosage while Harshan treated with one-half the dosage of doxycycline used in the current study. However, serological response rate seemed to be much lower in recently published studies treated with the same dosage of doxycycline used in the current study (19-21). The response rate was 63.4% and 82.9% in early syphilis in Tsai and Li’s study respectively. The broad range may be related to the different patient cohort in each
study, in addition to the different methods adopted by each study. Most studies
suggest a higher probability of serological failures for patients with more advanced
syphilis, HIV infection, and a history of syphilis (23-24). The difference could be
explained accordingly. On one hand, almost all the patients in our study were
HIV-Negative and without previous syphilis history, while in previous studies
HIV-infected patients accounted for 11% to 100% of the patients enrolled. If those
with HIV infection had increased risk of serological failure, this may have increased
the failure rate. On the other hand, the number of male patients in our study is similar
to female patients, most of these patients got married and they got syphilis treatment
together with their spouse, thus recurrent exposure to syphilis in the sexual network
and re-infection might be lower. Furthermore, our study mainly analysed the
patients completed 12 months follow up, patients lost to follow up were excluded.
Considering all the 163 intention-to-treat patients, the success rate would be much
lower (66.9%, 109/163).

There are few data about doxycycline treatment in late latent syphilis patients, our
study showed the response rate was 79.2%. Of note, most of the treatment failure
happened in patients with TRUST titer ≤ 8. For late latent syphilis, most patients had
received antibiotics for other conditions and the TRUST titer could have decreased
before syphilis specific treatment. In multivariate regression analysis, baseline
TRUST titer and syphilis stage were significantly associated with serological
response. Our findings generally support those of other investigations regarding the
relationship between the baseline RPR titres and the serological response (25). Only
two of these nine treatment failure patients shown relapse.

This study has several limitations. Firstly, Benzathine penicillin was not available in our hospital during the study period, the absence of a control group treated with benzathine penicillin G is a major concern. Secondly, the clinical data were collected from a single center and the study was retrospective, there were no standardized criteria to choose alternative therapy. Thirdly, the patients included in the study were those who had documented follow-up serological tests. The results were far from perfect because of the lost to follow up patients. Therefore, both selection and information biases should be acknowledged. Fourthly, some of the late latent syphilis were defined as unknown length of infection might be still in their early infectious stage, for those patients were with high titers of TRUST. The strengths of our study are that our study has a relatively large sample size of syphilis patients treated with doxycycline and late latent syphilis was included. Overall, our data, along with other reports, support the usage of doxycycline as a good alternative therapeutic option in the treatment of syphilis.

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Reference


Fig. 1 Patient flow chart.

Fig. 2 Serological response rate and Seroreversion rate at 12 months following treatment
Table 1 Demographic, clinical, and laboratory data of the syphilis patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>% (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56.8(67/118)</td>
</tr>
<tr>
<td>Female</td>
<td>43.2(51/118)</td>
</tr>
<tr>
<td>Serum TRUST</td>
<td></td>
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<tr>
<td>≤ 8</td>
<td>13.6(16/118)</td>
</tr>
<tr>
<td>16-32</td>
<td>39.8(47/118)</td>
</tr>
<tr>
<td>≥ 64</td>
<td>46.6(55/118)</td>
</tr>
<tr>
<td>Sexual orientation</td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>5.9(7/118)</td>
</tr>
<tr>
<td>heterosexual</td>
<td>94.1(111/118)</td>
</tr>
<tr>
<td>Stage of syphilis</td>
<td></td>
</tr>
<tr>
<td>primary</td>
<td>5.9(7/118)</td>
</tr>
<tr>
<td>secondary</td>
<td>54.2(64/118)</td>
</tr>
<tr>
<td>early latent</td>
<td>19.5(23/118)</td>
</tr>
<tr>
<td>late latent</td>
<td>20.3(24/118)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≤ 35</td>
<td>22.0(26/118)</td>
</tr>
<tr>
<td>36-50</td>
<td>36.4(43/118)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>41.5(49/118)</td>
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TABLE 2 Univariate analysis of factors associated with serological response at 12 months following treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR(95%CI)</th>
<th>Multivariate AOR(95%CI)</th>
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</thead>
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<tr>
<td>Sex</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.71 (0.44-6.73)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 35</td>
<td>0.50 (0.09-2.67)</td>
<td></td>
</tr>
<tr>
<td>35-50</td>
<td>0.87 (0.17-4.55)</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Serum TRUST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 16</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;32</td>
<td>28.33 (3.38-237.86) **</td>
<td>18.82 (2.14-165.90) **</td>
</tr>
<tr>
<td>Stage of syphilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>early</td>
<td>10.11 (2.31-44.21) **</td>
<td>5.14 (1.04-25.37) **</td>
</tr>
<tr>
<td>late</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Homosexual/bisexual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2.15 (0.23-20.07)</td>
<td></td>
</tr>
</tbody>
</table>

CI = confidence interval; OR = odds ratio; AOR = adjusted odds ratio; *P < 0.05; **P < 0.01
Syphilis patients received Doxycycline Treatment
N=163 (ITT)

3 didn’t complete doxycycline treatment

Patients completed Doxycycline treatment and 6 month follow up
N=129

Patients completed Doxycycline treatment and 12 month follow up
N=118 (PP)

Primary syphilis
N=7

Secondary syphilis
N=64

Early latent syphilis
N=23

Late latent syphilis
N=24
Figure 2