Molecular epidemiological survey of *Treponema pallidum* in pregnant women in the Zhabei District of Shanghai

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**Abstract**

**Purpose.** To evaluate the epidemiological characteristics of syphilis, such as incidence, vertical transmission and genotypes, in pregnant women in the Zhabei District of Shanghai. In addition, the changes in genotypes and the efficiency of genotyping were evaluated.

**Methodology.** We screened 3022 pregnant women for syphilis in the Shanghai Zhabei Central Hospital. Whole blood, plasma, earlobe blood and specimens from genital ulcers or skin/mucosal lesions were collected from syphilis-positive patients. Samples were genotyped by analysing a combination of three genes: *tpr, arp* and *tp0548* (the *tpr/arp/tp0548* genotype). Clinical data were further collected to evaluate disease incidence, maternal–neonatal transmission and social factors.

**Results.** Out of 3022 pregnant women screened for syphilis, 41 were syphilis-positive. Of these, 43.9 % showed vertical transmission (18/41). The prevalence of syphilis in pregnant women was 1.32 %, higher than that in other districts in Shanghai in 2014. Genotyping was performed in 10/11 (90.9 %) samples of syphilis lesions, 8/41 (19.5 %) blood samples, 12/41 (29.3 %) plasma samples, and 20/39 (48.7 %) earlobe blood samples. The predominant genotype was 14d/f, followed by 15d/f, 13a/f, 13d/f and 9o/c. Genotype 13a/f was reported for the first time in the Shanghai area since 2010.

**Conclusion.** The prevalence of syphilis in pregnant women in the Zhabei District was higher than that in other areas in Shanghai and the genotype is variable.

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**INTRODUCTION**

Syphilis is a sexually transmitted multi-stage disease caused by *Treponema pallidum*. Congenital syphilis is a significant public health problem in China. It has been reported that congenital syphilis has increased dramatically from 0.01 cases per 100 000 live births in 1991 to 69.9 cases per 100 000 live births in 2013, according to China’s national sexually transmitted disease surveillance system [1, 2]. The diagnosis of congenital syphilis is complicated by the fact that more than two-thirds of neonates are asymptomatic at birth and treatment delay can lead to permanent damage and disability [3]. Effective prevention and detection of congenital syphilis depends on the identification of syphilis in pregnant women. It is necessary to monitor all pregnant women during prenatal care and provide treatment as soon as possible to avoid the congenital infection.

In the Shanghai area in 2014, the incidence of syphilis was the highest among all Class B infectious diseases [4]. The Zhabei District, a national surveillance site for sexually transmitted diseases (STD), is an area of high incidence of syphilis in Shanghai.

According to the recommendations of the US Centers for Disease Control and Prevention (CDC), a combination of three genes, namely *arp, tpr* and *tp0548*, can be used to differentiate between strains of *T. pallidum* [5]. This makes it possible to the diversity of circulating subtypes of *T. pallidum*, to monitor changes in the prevalence and geographical distribution of the strains over time and to determine which new strains have been introduced in a specific area.

Results of studies of the molecular epidemiology of syphilis, performed in about patients infected with *T. pallidum*, have that the most predominant subtype of *T. pallidum* in Shanghai is 14d/f [6]. However, this trend was not consistent with the genotype distribution of *T. pallidum* in other regions of China. Moreover, the genotypic characteristics of *T. pallidum* isolated from pregnant women remain unclear [7, 8].
No genotypic studies of *Treponema pallidum* in pregnant women in Shanghai have been reported. In this study, serological tests for syphilis, genotyping and behavior monitoring were performed on pregnant women from the Shanghai Zhabei District Central Hospital, in order to investigate the characteristics and molecular epidemiological features of pregnant women with maternal syphilis.

**METHODS**

**Clinical specimens**

A total of 3143 pregnant women, recruited during their initial visit to the Zhabei District Central Hospital, from July 2013 to June 2015, were enrolled in the study and of them 3022 (3022/3143, 96.1 %) agreed to be screened for syphilis.

Venous blood (3 ml) was collected from the subjects and the rapid plasma reagin test (RPR, Shanghai Rongsheng Biotech) was performed in order to screen for syphilis. RPR-positive results were further confirmed by *T. pallidum* particle assay (TPPA; Zuhai Lizhu Biotech).

**Diagnostic criteria for syphilis**

The diagnostic criteria for syphilis during pregnancy are based on the Diagnostic Criteria for Syphilis [9] issued by the Department of Disease Control and Prevention, Ministry of Health of China, in August 2000. For pregnant women, a positive diagnosis of syphilis was confirmed if a subject showed positive results for both the RPR (titre >1:8 or an increase of four times the previous value) and TPPA tests. Congenital syphilis was confirmed when neonate showed both RPR-positive (titre increase four times the previous value) and TPPA-positive, TP-IgM positive. HIV infection of pregnant woman and newborns was diagnosed by Zhabei Disease Control and Prevention Center.

**Clinical specimen and data collection**

Venous blood (*n*=41) and blood plasma (*n*=41) were obtained from total blood with EDTA and collected from patients after informed consent was given. If a genital ulcer or a skin or mucosal lesion was present, exudate from mucosal lesions or genital ulcers (*n*=11) was collected by using a Dacron-tipped swab, and earlobe scrapings (*n*=39) were obtained from pregnant women with syphilis prior to the commencement of treatment with antibiotics.

Simultaneously, a survey was conducted to collect patient information using a standard sexually transmitted infection notification form in accordance with the National Sentinel Surveillance Program [10]. Relevant information on maternal syphilis was extracted by health staff from medical records. For each maternal syphilis case, data collected include information on demographic characteristics (e.g. maternal age, ethnicity, marital status, occupation, residency and education), clinical symptoms and stages, and knowledge of STD prevention and syphilis-infection-related information.

**Extraction of *T. pallidum* DNA**

*T. pallidum* DNA was extracted from the samples using the QIAamp DNA kit (Qiagen) according to the manufacturer’s protocol.

**Genotyping of samples**

The samples were screened for the *tp47* gene by a diagnostic PCR assay using an ABI 7500 thermal cycler (Applied Biosystems). The primers for the amplification of the *tp47* are as follows: forward primer 5’-CGTGTGGTATCAAATCTGG-3’, reverse primer 5’-TCAACGTGTACTAGTGC-3’.

In samples testing positive, the *arp*, *tp* and *tp0548* genes were then amplified. The genotype of each sample is denoted as *arp-trp-tp0548*.

The *arp* gene contains 2–22 repeated sequences of 60 bp. The *T. pallidum* *arp* type may be classified into a total of 21 subtypes based on the number of repeats. The primers for the amplification of *arp* are as follows: forward primer 5’-CAAATCGAGGACTGTC-3’, reverse primer 5’-GGTACATCCGTGGAT-3’. The concentration of primer used for the amplification of *arp* was 0.4 mol l\(^{-1}\); 10 µl of DNA was amplified using 2.5 units GoTaq Flexi DNA polymerase (Promega) in a 50 µl reaction with 200 mM dNTPs, 0.6 µM primers, 1.5 mM MgCl\(_2\) and 1× GoTaq Flexi Buffer. Reaction conditions were: 94°C for 4 min, 40 cycles at 94°C for 1 min, 61°C for 1 min, and 72°C for 2 min 30 s, with a 15 min extension at 72°C. The size of the amplified gene fragment and the number of repeats were analysed using Image Lab software.

*tp* (*E*, *G* and *J*) genotyping was performed using an RFLP method, as described by Lorenzo Giacani et al. [11]. Currently, 16 subtypes of *T. pallidum* tpr, namely a–p, are known. The primers for the amplification of *tpr* were as follows: forward primer 5’-CAGTTTTTGGCCGTAAAGC, reverse primer 5’-AACATGAGGAGAATAGTC-3’. The concentration of the primer used for amplification of the *tpr* gene was 0.6 mol l\(^{-1}\).

A 5 µl sample of DNA was amplified using 2.5 units of GoTaq Flexi DNA polymerase in a 50 µl reaction with 200 mM dNTPs, 0.6 µM primers, 2.5 mM MgCl\(_2\) and 1× GoTaq Flexi Buffer. PCR conditions were the same as those used for the amplification of *arp*. PCR products were digested with the restriction endonuclease *MseI* (Invitrogen) and resolved via electrophoresis on a 2% agarose gel. The RFLP method was utilized to determine subtype.

*tp0548* genotype was determined based on the *T. pallidum* open reading frame sequence 131–215. The primers for the amplification of *tp0548* are as follows: forward primer 5’-GTCCTATATGATCTGTTTCC-3’, reverse primer 5’-GTATGGGTCTGCGATTGG-3’. For amplification from isolates, 5 µl of DNA was amplified using 2.5 units of GoTaq Flexi DNA polymerase in a 50 µl reaction with 200 mM dNTPs, 0.8 µM primers, 1.5 mM MgCl\(_2\) and 1× GoTaq Flexi Buffer. PCR conditions were as follows: 95°C for 2 min, 40 cycles 95°C for 1 min, 62°C for 1 min and 15 s,
and 72°C for 1 min, extension for 10 min at 72°C. The amplified products were sequenced and compared with the standard sequence to determine the subtype. Subtypes c–g and i were identified.

Gene primers used for the amplification of \textit{tp47}, \textit{arp}, \textit{tpr} and \textit{tp0548}, as previously described [12], were synthesized by Sangon Biotech.

### Statistical analysis

Epi-data software, developed by the Shanghai Comprehensive HIV Surveillance Program, was utilized for data entry. SPSS 16.0 was used for statistical analysis. The $\chi^2$ test was used and results with $P$ values below 0.05 were considered statistically significant.

### RESULTS

#### Demographic characteristics of the pregnant female subjects demographic data are given in Table 1

A total of 3022 pregnant women were screened over a 2-year period; 41 showed positive results for the RPR and TPPA tests. The total positive rate was 1.36%. From July 2014 to June 2015, the positive rate (1.51%) was higher than that between July 2013 and June 2014 (1.23%). The pregnant women who tested positive for RPR and TPPA were mostly in the age ranges 20–29 years (68.3%) and 30–39 years (26.8%), with a mean age of 28.3 years (28.3±5.1). Most of the confirmed cases were latent syphilis, accounting for 73.1% (30/41) of the total cases. As regards education and employment, 82.9% (34/41) of the women were educated to below associate-degree level, and 48.2% (20/41) did not hold a job.

#### Characteristics of childbirth and vertical transmission in pregnant women testing positive for syphilis

The details of childbirth and congenital syphilis occurrence in pregnant women with syphilis infections are listed in Table 2.

As shown in Table 2, a high level of occurrence of premature birth or stillbirth was observed in pregnant women with syphilis. Seven out of 41 (17.1%) patients underwent premature delivery or stillbirth compared with 10% overall in China, which has been reported for 2012 by the World Health Organization (WHO) [13]. A total of 18 newborns were syphilis-positive, and the vertical transmission rate was 43.9% (18/41). None of the neonates was positive for HIV infection.

#### Genotyping of DNA from the clinical specimens

Out of a total of 41 whole-blood samples, DNA was extracted from 13 samples (13/41, 31.7%) and 8 samples were genotyped. Out of a total of 41 blood plasma samples, DNA was extracted from 17 samples (17/41, 41.45%) and 12 samples were genotyped. Out of 39 earlobe blood samples,
DNA was extracted from 25 (25/39, 64.1%) and 21 samples were genotyped. Out of a total of 11 samples of mucus lesions, DNA extraction and genotyping were performed for 10 samples. Results of genotyping for the 21 patients are shown in Tables 3, 4 and 5.

Four arp genotypes (Fig. 1) (subtypes 9, 13, 14 and 15) were identified in the specimens. Three RFLP types (subtypes a, d and o) were identified within the tpr II subtype (Fig. 2). Two types, subtypes c and f, were found for tp0548, with 90.5% (19/21) being subtype f. The 14d/f (17/21, 80.9%) genotype was the most commonly identified genotype in the analysed clinical samples, followed by one case (1/21, 4.7%) each of the 15d/f, 13a/f, 13d/f and 9o/c genotypes. The predominant genotype in Shanghai is 14d/f. As shown in Figs 1 and 2.

**DISCUSSION**

In the present study, 41 out of 3022 pregnant women showed positive results for both RPR and TPPA tests, with a positive rate of 1.36%. The disease prevalence in 2015 (20/1321, 1.51%) was observed to be slightly higher than in 2014 (21/1701, 1.23%) (P>0.05). Furthermore, in 2014 and 2015, the incidence of syphilis in pregnant women in the Zhabei District was higher than 70.73% [4] which was reported by Shanghai Municipal Commission of Health and Family Planning the Shanghai area in 2014.

In pregnant women, syphilis infections are mostly latent in nature [14, 15]. Accordingly, in this study, 73.1% (30/41) of the identified cases were latent syphilis. The infected pregnant women were mainly between 20 and 29 years of age, accounting for 68.83% (28/41) of the total incidence of syphilis, followed by the 30–39-year-olds, who accounted for 26.83% of the total incidence. A large floating population is present near the train station in the Zhabei District, in which the incidence of STD is high. The incidence of syphilis in pregnant women is significantly higher in Zhabei than that in Shanghai. In our survey, most syphilis-infected pregnant women showed a lack of knowledge of STD prevention, and a correlation was found between the occurrence of syphilis in pregnant women and high migration rate, low education level, and lack of formal employment compared with the enrollment group. In the current study, 82.9% (34/41) of the syphilitic pregnant women were without university education, 48.78% (20/41) were not formally employed, and 53.7% (22/41) were from provinces other than Shanghai, compared with 38.5% (1164/3022) of the enrollment group. Our findings indicate that it is crucial to provide information regarding STD and increase awareness of syphilis among pregnant women.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number</th>
<th>Whole blood</th>
<th>Plasma</th>
<th>Earlobe blood</th>
<th>Lesion</th>
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<tbody>
<tr>
<td>Stage 1</td>
<td>8</td>
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<td>4/8 (50%)</td>
<td>6/8 (75%)</td>
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<td>2/3 (66.7%)</td>
<td>2/3 (66.7%)</td>
<td>3/3 (100%)</td>
<td>3/3 (100%)</td>
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<tr>
<td>Latent</td>
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<td>8/30 (26.7%)</td>
<td>11/30 (36.7%)</td>
<td>16/28 (57.1%)</td>
<td>10/11 (90.9%)</td>
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<tr>
<td>Total</td>
<td>41</td>
<td>13/41 (31.7%)</td>
<td>17/41 (41.5%)</td>
<td>25/39 (64.1%)</td>
<td>10/11 (90.9%)</td>
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<table>
<thead>
<tr>
<th>Stage</th>
<th>Whole blood</th>
<th>Plasma</th>
<th>Earlobe blood</th>
<th>Lesion</th>
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<td>3/4 (75%)</td>
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<td>2/2 (100%)</td>
<td>3/3 (100%)</td>
<td>3/3 (100%)</td>
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<tr>
<td>Latent</td>
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<td>7/11 (63.6%)</td>
<td>13/16 (81.3%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>8/13 (61.5%)</td>
<td>12/17 (70.6%)</td>
<td>21/25 (84%)</td>
<td>10/10 (100%)</td>
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<table>
<thead>
<tr>
<th>Stage</th>
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<th>15d/f</th>
<th>13a/f</th>
<th>13d/f</th>
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<tr>
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<td></td>
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<tr>
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In this study, no HIV infection was found among the pregnant women with syphilis, which may be attributable to the HIV prevention measures implemented in the Zhabei District in recent years [16].

The present results of T. pallidum genotyping differ slightly from those described in previous reports [8, 17]. Of the 21 patients who had sufficient DNA for the organism to be subtyped, 14d/f (17/21, 80.9%), the predominant genotype in the Shanghai region, was the most commonly observed genotype in the clinical samples. In addition, one case (1/21, 4.7%) each of genotypes 15d/f, 13a/f, 13d/f and 9o/c was identified. The predominant genotype identified in the current study was 14d/f, which is consistent with the findings of similar studies from 2007 to 2011 [18, 19]. A small sample epidemiology survey in about 80 patients infected with T. pallidum in 2012 in the Shanghai area [19] failed to identify the 13a/f subtype, which is a relatively rare subtype. The present identification of subtype 13a/f, which is the first reported case since 2010, may be attributed to the high migration rate in Zhabei District. People migrating from other places may have a different subtype of T. pallidum which is common in those places.

In the current study, blood samples were found to show a lower DNA extraction rate compared with samples from skin and mucosal lesions, which is consistent with the results described in previous reports [18, 20]. It has been shown that this is mainly due to the higher load of T. pallidum in the skin and mucous membranes than in the blood [21, 22]. The presence of relatively high concentrations of PCR-inhibiting material in whole-blood samples may represent an additional contributing factor.

The genotyping efficiency of DNA extracted from earlobe blood samples was higher than that of DNA from whole blood and blood plasma. Its genotyping efficiency is similar to that for samples from mucosal lesions, possibly due to the high density of capillaries in the earlobe. Castro et al. [23] showed that, in patients with latent syphilis, T. pallidum may be present in the capillary bed; this is considered to contribute to the high genotyping efficiency of DNA extracted from earlobe blood. The earlobe is a highly suitable site for blood sampling due to the relative lack of sensory nerves in this region. Additionally, the high efficiency of genotyping of DNA from blood collected from the earlobe indicates that this site is ideal for sampling in patients with latent syphilis and no obvious lesions. Therefore, our findings indicate that earlobe blood sampling may be highly useful for the molecular diagnosis and genotyping of T. pallidum infections. However, few studies have been performed to date [22], our investigation is only about the Zhabei District of Shanghai and used a small sample, and further investigation is required in order to confirm our findings.

Conclusions

In summary, we have characterized the incidence and genotype of T. pallidum in pregnant women in the Zhabei District of Shanghai. The prevalence was higher than that in other areas in Shanghai and the genotype was variable. To our knowledge, this is the first molecular epidemiological survey for T. pallidum in pregnant women in the Shanghai area.

Funding information

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The Institutional review board (IRB) of Shanghai Zhabei Central Hospital gave ethical approval. The IRB approval number is ZBLL2014061202.

References


