Effect of False-Positive Syphilis Spirochete-Specific Antigen on Pregnancy

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LW and FF contributed equally to this work as co-first author. LW and FF contributed to the design and interpretation of the current study. KP, YZ and LZ conducted the experiments in this study. All authors wrote, revised, and approved the final manuscript.

Key words
Syphilis screening, Pregnancy, Chemiluminescent immunoassay, Spirochete, False positive syphilis, Pregnancy outcome

ABSTRACT
Chemiluminescent immunoassay (CIA) has been suggested as a screening test for syphilis. The potential clinical value of the false positive result of obstetric CIA screening has not been elucidated. Our study aims to investigate the possible association between CIA false-positivity for syphilis and pregnancy outcome, which may provide information for obstetric clinical management. We retrospectively investigated the singleton pregnant women who had delivered at our center, and screened for syphilis by using CIA during early pregnancy from January 1, 2010 to December 31, 2019. Pregnancy outcomes of syphilis false-positive group were compared with those of syphilis negative group. Total of 16,935 singleton pregnant women were included in present study Compared with negative group, false-positive group shows older delivery age, smaller gestational weeks at delivery, and lower birth weight (P<0.05). Besides, the incidences of gestational hypertension, gestational diabetes, immune system diseases, and preterm delivery were also higher than those of negative group (P<0.05). Our study indicates that the pregnancy outcomes of syphilis false-positive group are poor, suggesting its relevance to obstetric prognostic and may need more attention from early pregnancy period, including antenatal examination and necessary laboratory tests to exclude other systemic diseases.

INTRODUCTION
Syphilis is a chronic bacterial infection caused by the spirochete Treponema pallidum (TP) which is highly contagious, and can be transmitted through sexual contact or across the placenta during pregnancy (Eppes et al., 2022). Almost all cases of syphilis are transmitted through sexual contact, mother to fetus or blood transfusion (Waheed et al., 2017).

The prevalence of this disease varies in different societies and years, and the most common test that is used as the first step to diagnose this disease is the venereal disease research laboratory (VDRL) test, which is a non-treponemal standard test and used as a simple and inexpensive diagnostic method to detect syphilis in people suspected of having the disease at first and then to control the treatment (Young, 2000). Unfortunately, this test is false negative in 25-30% of early latent and delayed stages of syphilis, and it is false positive in many cases, including viral diseases or their vaccine injection, genital herpes, HIV, malaria, intravenous drug injection, old age, autoimmune diseases, lupus erythematosus and rheumatoid arthritis and pregnancy. This test is about 100% positive only in the second stage of the disease (Harris et al., 2001). For this reason, this test is not very valuable in primary syphilis, which is positive in 78% of cases, and in secondary syphilis, which is 71% of cases (Nelson et al., 2004).

The diagnosis and detection of syphilis is essential to prevent transmission and control the epidemic, therefore all pregnant women are required to take syphilis screening at the first prenatal visit or at first presentation to care in China (Qiao et al., 2020). In the past, prenatal screening for syphilis was performed with a nontreponemal test such as the rapid plasma reagin (RPR) test, toluidine red unheated serum test (TRUST) assay etc. At present, chemiluminescence immunoassay (CIA) of Syphilis TP has outcompeted the nontreponemal tests with the advantage of specificity, high sensitivity, rapidity and automation. It is worth noticing that the use of the CIA-based algorithm results in identification of patients with false positive results, especially in pregnant women (Wang et al., 2016). The objective of present analysis was to describe outcomes
among singleton pregnant women who were screened as syphilis false positive (CIA+/TRUST-/TPPA-). Our study may provide a basis for clinical management of such group of pregnant women.

MATERIALS AND METHODS

Sample
We retrospectively investigated the singleton pregnant women who had delivered at our center from January 1, 2010 to December 31, 2019. Each one was screened for syphilis by CIA at the beginning of pregnancy and those with false positive (CIA+/TPPA-/TRUST-) and negative (CIA-) results were included into present study. Pregnant women who had spontaneous abortion and intrauterine death during pregnancy were excluded from our study.

Syphilis testing procedure
All cases during their first prenatal visit were serend by TP-CIA. CIA is a syphilis spirochete-specific antibody assay which was performed by using Abbott Architect Syphilis TP Reagent Kit, with the detection of ARCHITECT i2000SR chemiluminescent microparticle immunoassay analyzer according to the instructions. A titer of less than 1 was considered negative and more than 1 was considered positive. Those cases which were CIA-positive were further checked. Syphilis toluidine red unheated serum test (TRUST), a non-specific antibody test for syphilis spirochetes. The reagents were purchased from Shanghai RongSheng Biological Pharmaceutical Co. The operation was carried out strictly in accordance with the kit and instrument instructions, and negative and positive controls were performed. The patients with discordant serological results (CIA+/TRUST-) were be further checked by T. pallidum particle agglutination assay (TPPA): This is a specific antibody test for T. pallidum, and the reagents are manufactured by Fuji Co. Japan or Rong Sheng Co. Shanghai.

Study variables
Maternal delivery age, gravida, gestational week of delivery, birth weight, the incidence of gestational hypertension, gestational diabetes, immune system disorders, premature rupture of membranes, preterm delivery, and small for gestational age in syphilis false-positive and negative groups were analyzed.

Data management and statistical analysis
Statistical analyses were performed using SPSS 20.0. Measurement data were expressed as mean ± standard deviation, and t-test and non-parametric test were used for statistical analysis. Numerical data were expressed as percentages, and χ2 test and Fisher’s exactness test were used. P < 0.05 was considered statistically significant.

RESULTS

Table I shows the results of syphilis screening during pregnancy. Among the 16935 pregnant women who had regular prenatal examination and delivered at our center from January 1, 2010 to December 31, 2019, 219 cases were detected as CIA-positive group, and 16717 cases were CIA-negative group which were included as the control group in present study, accounting for 98.71% of total (16717/16935). The CIA-positive cases were rechecked by TRUST, and 4 of them were positive, consistent with CIA test while the rest of them (215 cases) were TRUST-negative cases. The CIA+/TRUST- cases were then further checked by TPPA, and 68 cases among them were confirmed as TPPA-positive cases while the rest (147 cases) were TPPA-negative group (CIA+/TRUST-/TPPA). The false-positive rate of CIA test was 67.12%.

Table I. Results of syphilis screening of 16935 pregnant women.

<table>
<thead>
<tr>
<th>Results</th>
<th>n</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIA+/TRUST+</td>
<td>4</td>
<td>Syphilis (active)</td>
</tr>
<tr>
<td>CIA+/TRUST-/TPPA+</td>
<td>68</td>
<td>Syphilis (past or active)</td>
</tr>
<tr>
<td>CIA+/TRUST-/TPPA-</td>
<td>147</td>
<td>False positive</td>
</tr>
<tr>
<td>CIA-</td>
<td>16717</td>
<td>No syphilis</td>
</tr>
</tbody>
</table>

Table II shows the comparison of pregnancy outcomes between the false positive group and the control groups. Comparison of maternal delivery age, gravida, gestational week of delivery and birth weight between the two groups. Delivery age and birth weight were normally distributed and compared between groups by t-test; gestational age and gestational week of delivery were not normally distributed and compared between groups by non-parametric test. The false positive group had smaller gestational week and lower birth weight, all of which were significantly different (P < 0.05), while there was no significant difference in maternal delivery age and gravida between two groups (P >0.05).

Table III shows the Comparison of the incidence of gestational hypertension, gestational diabetes, immune system disorders, premature rupture of membranes, preterm delivery, and small for gestational age between two groups. Among 147 false-positive cases, the incidences of gestational hypertension, gestational diabetes, immune system disorders, premature rupture of membranes, preterm
Table II. Comparison of pregnancy outcomes.

<table>
<thead>
<tr>
<th></th>
<th>False positive group</th>
<th>Negative group</th>
<th>T/Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery age</td>
<td>29.35±4.36</td>
<td>29.59±3.42</td>
<td>0.67</td>
<td>0.51</td>
</tr>
<tr>
<td>Gravida</td>
<td>1.60±0.97</td>
<td>1.70±0.98</td>
<td>-0.84</td>
<td>0.40</td>
</tr>
<tr>
<td>Delivery gestational week</td>
<td>36.35±5.50</td>
<td>39.14±4.10</td>
<td>-13.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Birth weight</td>
<td>2982.30±605.84</td>
<td>3374.90±605.84</td>
<td>7.30</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table III. Comparison of some of perinatal outcomes.

<table>
<thead>
<tr>
<th>Pregnancy outcomes</th>
<th>False positive group n (%)</th>
<th>Negative group n (%)</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational hypertension</td>
<td>16(10.88)</td>
<td>307(1.84)</td>
<td>63.53</td>
<td>0.000</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>27(18.36)</td>
<td>1417(8.47)</td>
<td>18.22</td>
<td>0.000</td>
</tr>
<tr>
<td>Immune diseases</td>
<td>27(18.37)</td>
<td>0(0.00)</td>
<td>2391.69</td>
<td>0.000</td>
</tr>
<tr>
<td>Premature rupture of membranes</td>
<td>22(14.97)</td>
<td>2259(13.51)</td>
<td>0.26</td>
<td>0.347</td>
</tr>
<tr>
<td>preterm delivery</td>
<td>65(44.22)</td>
<td>684(4.09)</td>
<td>553.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>8(5.44)</td>
<td>547(3.27)</td>
<td>2.16</td>
<td>0.112</td>
</tr>
</tbody>
</table>

delivery and small for gestational age were 10.88 % (16/147), 18.36 % (27/147), 18.37 % (27/147), 44.22 % (65/147), respectively, which are significantly higher than those of the control group (P< 0.05). As for the incidence of premature rupture of membranes and small for gestational age between the false-positive and negative groups, there shows no difference (P> 0.05).

**DISCUSSION**

Congenital syphilis has increased substantially as the rates of syphilis infections are on the rise during the last 20 years (Uku et al., 2021). Syphilis screening is very important to reduce its spread. Serodiagnostic tests are the only means for screening asymptomatic individuals. There are two categories of serodiagnostic tests: Nontreponemal tests (NTTs) and treponemal tests (TTs). NTTs measure immunoglobulins (IgM and IgG) produced in response to lipoidal material released from the bacterium and/or dying host cells. They are useful in detecting active syphilis and often used in treatment monitoring. In contrast to NTTs, TTs detect antibodies directed against T. pallidum proteins, such as CIA, TPPA etc., which are highly specific tests for syphilis. CIA has been commercialized, and it is widely used in large-scale screening for the advantage of sensitivity, automation and objective readout.

However, the high sensitivity brings CIA the problem of false positivity. A retrospective analysis of syphilis screening results in blood donors by Sandes et al (2017) in Brazil shows a false-positive rate of 37.4 % for CIA testing, which indicates that CIA for syphilis screening in healthy populations may have a high false-positive rate. Another study by Mmoge et al. (2015) in the United States shows that up to 80 % of pregnant women who tested positive by CIA were false positive. Our analysis shows the syphilis false-positive rate of the CIA test in our center is 67.12%. With such a high false positive rate, is CIA suitable for syphilis screening in pregnancy? Laboratory results from Beijing Friendship Hospital in China showed that the sensitivity of the CIA for syphilis screening was 100 %, the specificity was 99.8%, and the false-positive rate was only 0.22%. When the threshold value was set above 10, the true-positive rate of the test could reach 100%. Compared with the sensitivity (65%), specificity (99.6%) of RPR, CIA was recommended for syphilis screening (Wang et al., 2016). Similar results were also reported by Adhikari et al. (2020) in the United States with Abbott’s CIA test for RPR (+) pregnant women. They show the CIA sensitivity of 100%, specificity of 99.9%, positive predictive value of 97.4 %, and a false positive rate of only 0.06%.

Williams et al. (2020) suggest that screening for syphilis in pregnancy using non-specific syphilis spirochetal antibody tests has a higher rate of false positives and can lead to clinical over-diagnosis and treatment. Therefore, most laboratories, including our center, switched from the traditional non-specific syphilis antibody test to specific syphilis antibody test for syphilis screening, and the positive ones are reflexed to non-specific syphilis antibody test. Although the CIA test shows a high false positive rate in pregnancy, its high sensitivity helps to avoid missed diagnosis. This can effectively reduce the occurrence of congenital syphilis, and it is more convenient, efficient and cost effective using this protocol for pregnancy screening.

Most of these false-positive cases show no clinical
symptoms and signs, no history of syphilis infection, or exposure history. However, the results of CIA false positive not only caused psychological stress and panic of the pregnant women, but also brought heavier burden of explanation to the medical services. Besides the specific syphilis antibody, are there any other factors interfering the CIA results? A composition analysis of the false positive cases of CIA at Beijing Friendship Hospital in China revealed that the majority of the cases are composed of pregnant women, while the rest include the aged, tumor patients, and hemodialysis patients in-sequence (Wang et al., 2016). The correlation between serological false positives and age, presence of malignancy, rheumatic immune system diseases, and infectious diseases was also supported by others’ studies. Bian et al. (2017) have reported that the false positive rate of syphilis specific antibodies in elderly patients tends to increase with age. The study of He et al. (2017) shows that antinuclear antibodies would interfere with the detection of syphilis antibodies in pregnant women, resulting in false-positive results. Swain and Riordan (2020) in the UK reported a case of a 25-years-old pregnant woman who developed a false-positive syphilis result after receiving immunoglobulin therapy, and the infant also tested false-positive. False positives for syphilis have also been detected in children with adenoid hypertrophy (Shi et al., 2018). All the above diseases or conditions show common feature of abnormal immunity, which may suggest that CIA false positive for syphilis screening in pregnant women might be related to the presence of some immune substances cross-reacting with the specific syphilis antigen. Therefore, the high rate of false positives during pregnancy may relate to the altered immune status during pregnancy. Furthermore, our findings show that the incidence of immune system diseases in the group of false negative pregnant women is higher than that of the control group (18.73% vs 0%), supporting that autoimmune antibodies associated with immune system disorders can interfere with CIA testing and lead to false-positive results.

Our study shows that the CIA false-positive rate of syphilis in pregnancy is 67.12%, and the false-positive cases had a greater risk of getting gestational hypertension, gestational diabetes, immune system disorders and preterm birth than the CIA negative group, in addition the average newborn weight of CIA false positive group was lower than that of the control. As early as 1987, there was report indicating that pregnant women of syphilis false positive had poor pregnancy outcomes (Thornton et al., 1987). De Carolis et al. (2018) reported that a statistically significant lower neonatal birth weight was observed in women with false-positive TORCH associated with antiphospholipid antibodies positivity in comparison with those in women with false-positive TORCH without antiphospholipid antibodies positivity. It is well known that immune system disorders such as antiphospholipid syndrome can lead to spontaneous abortion, stillbirth, and low birth weight. Previous studies also indicate that false positive syphilis in pregnancy is associated with immunity, which call attention to us that we should pay more attention to this group of pregnant women, especially in investigating whether there is a comorbidity of immune system disorders. In this way, we may achieve early detection and treatment, and improve pregnancy outcome.

The shortcomings of our study: First, our center sets CIA test titer greater than 1 as positive, and pregnant women with titers between 1 and 9 maybe have a greater possibility of false positives (Fan et al., 2014). It is necessary to further expand the sample size in future studies to determine the threshold value of CIA titer which may help to reduce the false positive rate. Second, there were 27 cases of immune system diseases in syphilis false positive group, while that in syphilis negative group was 0. As immune system diseases are not included in routine screening of all pregnant women unless they have clinical manifestations. Therefore, we are actually not sure the accurate figures of the cases combined immune system diseases in both groups.

CONCLUSION

Our study shows that false-positive rate of CIA syphilis screening during pregnancy is high (> 50 %), and this CIA false-positive shows obvious relevance to the incidence of perinatal adverse outcomes. These finding alerts obstetricians to pay more attention to this group of cases from early pregnancy, choose the necessary laboratory tests for these cases to exclude medical complications, especially immune system diseases, and accordingly increase the frequency of perinatal examinations to minimize the adverse pregnancy outcomes.

As a matter of fact, all pregnant women in our center are required to be screened for immune system diseases for CIA false positive women. In the follow-up study, we will work together with our Department of Laboratory Medicine to determine the optimum threshold values for CIA testing in order to reduce the false-positive rate of CIA, as well analyze the possible factors associated with false-positive syphilis tests to provide evidence-based medical clue for obstetric management.

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IRB approval

This research was carried out with the approval of Characteristic Medical Center of Strategic Support Forces and informed consents were signed by the patients and/or guardians.

Ethical approval

The study was approved by the ethics committee of Characteristic Medical Center of Strategic Support Forces and informed consents were signed by the patients and/or guardians.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES


