Performance of Chemiluminescent Microparticle Immunoassay in Screening for Syphilis in Pregnant Women from Low-Prevalence, Resource-Limited Setting

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Objective: *This study evaluated the diagnostic performance of chemiluminescent microparticle immunoassay (CMIA) in screening for syphilis in Asian pregnant women.*
Material and Method: *This study retrospectively reviewed the CMIA results of pregnant women attending the antenatal care clinic, King Chulalongkorn Memorial Hospital. Women with reactive CMIA results were extracted from the laboratory database and further analyzed. A reactive/positive result for Treponema pallidum was defined as having a sample/cut-off absorbance ratio of ≥1.0. Samples were also tested by rapid plasma reagin (RPR) and Treponema pallidum particle agglutination assay (TPPA).*
Results: *From February 2011 to January 2013, a total of 11,640 pregnant women were tested and 65 women (0.56%) had reactive CMIA results. Among these cases, 58 women (89.2%) had non-reactive RPR results. TPPA were non-reactive in 35 women (60.3%) who had non-reactive RPR results. A total of 23 women (39.7%) with RPR non-reactive and TPPA reactive results; therefore, the prevalence rate of syphilis in this population was estimated as 1.98 per 1,000 pregnant women. Among this, 7 cases had a history of past, partial treatment for syphilis and 16 cases were considered as untreated, late, latent syphilis. If RPR tests were used as the screening test, 16/23 cases (69.6%) cases with untreated syphilis would be missed.*
Conclusion: *Even though CMIA has high false positive results, however it is still recommended that this reverse sequence screening be used instead of the traditional algorithm. The rate of false positive results can be decreased by adjusting the sample/cut-off absorbance ratio of CMIA.*

Keywords: *Chemiluminescent microparticle immunoassay, Pregnancy, Rapid plasma reagin, Syphilis screening, Treponema pallidum particle agglutination assay*

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Syphilis is caused by *Treponema pallidum*, a spirochete, which cannot be cultured so for most cases, serological tests are used to diagnose this infection. Since syphilis infection can be asymptomatic, therefore it is recommended to use serologic screening to detect latent infections in high-risk population such as blood donors and pregnant women to prevent transmission by blood transfusion and mother-to-child transmission respectively.

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If syphilis is left untreated in pregnant women, the vertical transmission of the bacteria will result in congenital syphilis, and cause various complications such as late abortion, still birth, preterm delivery, and low birth weight. These adverse outcomes can be prevented through early detection and treatment of syphilis during pregnancy[1]. Hence, the World Health Organization (WHO) has recommended that serological screening tests should be performed on all pregnant women at their first antenatal care visit and be repeated again in the third trimester[2].

Traditionally, non-treponemal tests, such as the rapid plasma reagin (RPR) test or the Venereal Disease Research Laboratory (VDRL) test, are used to serologically screen for syphilis. A reactive non-treponemal test is confirmed by using any one of the

treponemal tests: the *Treponema pallidum* particle agglutination (TPPA) test or the fluorescent treponemal antibody absorbed (FTA-ABS) test[3]. Recently, laboratories that receive a large number of samples no longer follow the traditional algorithm for syphilis screening with a non-treponemal test followed by a treponemal test. Instead, the new algorithm now screens with a treponemal test, either the enzyme immunoassay (EIA) or the chemiluminescent microparticle immunoassay (CMIA), followed by a non-treponemal test for confirmation[3-5]. This is done in order to keep up with the number of samples received per day at the hospital laboratories, CMIA can process up to 400 samples per hour by automated machine while RPR or VDRL is a manual test that required medical technologist and with limited capacity of ~20 tests per hour. Likewise, the King Chulalongkorn Memorial Hospital (KCMH), a tertiary center in Bangkok, Thailand, has changed its algorithm for syphilis screening in February 2011 to CMIA as its initial screening test to keep up with the times.

Even though EIA or CMIA has several advantages such as high throughput and high sensitivity to detect syphilis infection, however one of its problems is the high yield of false positive results[6-9]. This is of concern since a recent report demonstrated that the prevalence of syphilis in Asian pregnant women screened by traditional screening algorithm was only 0.14%[10]. In order to ensure that the new algorithm is not inferior in screening for syphilis in a high-risk population in a low prevalence setting, this study evaluated the diagnostic performance of CMIA as the initial syphilis-screening test in Asian pregnant women attending the antenatal care clinic at the KCMH.

Material and Method

This is a retrospective, descriptive study of CMIA's performance in pregnant women. CMIA data of all pregnant women attending the antenatal care clinic at the KCMH were extracted from the database of the Department of Microbiology from February 2011 to January 2013. Results from the first visit and the third trimester were extracted and analyzed. For those women with reactive CMIA results, their medical records were further reviewed to collect the demographic and other pertinent, clinical data.

The procedure for testing the samples at the antenatal clinic begins with the immediate delivery of the specimens at room temperature after or within 24 hours of collection to the Immunology laboratory of the Department of Microbiology, Faculty of Medicine, Chulalongkorn University. The specimens were tested by the ARCHITECT Syphilis TP (Abbott Laboratories. Abbott Park, Illinois, USA). The CMIA for *T. pallidum* uses microparticles coated with the three recombinant antigens (TpN15, TpN17, TpN47) and acridinium-labelled anti-human IgG and IgM monoclonal antibodies were used as conjugates. The end result of chemiluminescence was measured as relative light units (RLUs). Specimens yielding RLUs less than the cut-off were considered negative; specimens with RLUs greater than the cut-off are considered positive[11]. The sample/cut off value (S/CO) $\geq$1.00 was considered reactive as suggested by the manufacturer. Serum with reactive CMIA result was subsequently tested with the secondary assays such as the RPR and TPPA. Results were entered into the laboratory database. This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University.

Women with reactive CMIA results were divided into three groups (Fig. 1). The first group was composed of women with RPR reactive and TPPA reactive results and were considered as having active syphilis infection and were treated with the standard regimens. The second group was composed of women with RPR non-reactive and TPPA non-reactive results and were considered as false positive CMIA. The third group was composed of women with RPR non-reactive and TPPA reactive results and were considered as past treated syphilis, primary infection or untreated, late, latent syphilis. For the latter group, the history of treatment was used to further classify the women into

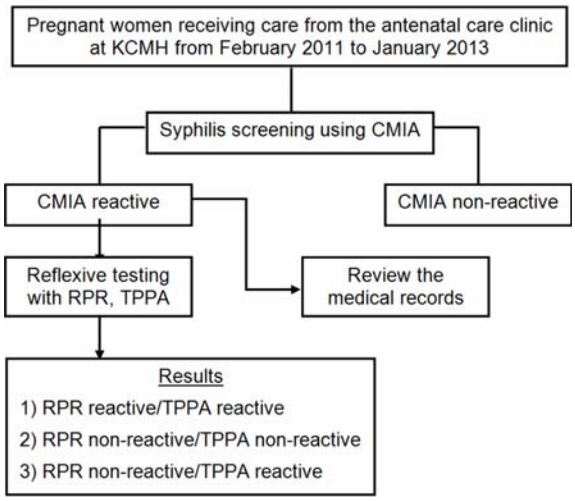

Fig. 1 Flow chart of the study.

treated, primary and latent subgroups. Baseline characteristics and demographic data of CMIA reactive women were presented as mean and standard deviation (SD), median and range, and percentage as appropriate.

Results

From February 2011 to January 2013, a total of 11,640 pregnant women were screened with CMIA for *T. pallidum* at the first antenatal visit and were included in the analyses. Among these women, 65 cases (0.56%) had reactive test results. The demographic data of CMIA reactive women are shown in Table 1. Mean age of CMIA reactive women was 32.1 years and most of them had no underlying disease. One pregnant woman had asymptomatic HIV infection and three other women had asymptomatic hepatitis B viral infections. Nine pregnant women had previously treated for syphilis. Among 65 women, 50 women delivered at KCMH and 15 women had no delivery data. There were two still birth fetuses. The first case was cesarean delivered at 33 weeks of gestation due to maternal intrapartum eclampsia. Severe hydrops fetalis with no significant anomaly was noted in the pathological report. The other case was recorded as a fetal death in utero at 39 weeks of gestation and the autopsy results did not identify the definite cause of death.

Table 1. Demographic data and fetal outcome of women with reactive CMIA

Characteristics	n = 65
Age (years)*	32.1±7.4
Gravida**	2 (1-6)
Gestational age at screening (weeks)**	13 (5-38)
Occupations***	
Employee	34 (52.3)
Housewife	17 (26.2)
Business	6 (9.2)
Government officer	2 (3.1)
Others	6 (9.2)
Underlying disease***	
None	54 (83.1)
Hepatitis B viral infection	3 (4.6)
Anemia	2 (3.1)
Diabetes mellitus	1 (1.5)
HIV infection	1 (1.5)
Others	4 (6.2)
Fetal outcome***	
Abortion	4 (6.2)
Stillbirth	2 (3.1)
Preterm live birth	2 (3.1)
Term live birth	42 (64.6)
Unknown	15 (23.0)

Data presented as * mean ± SD, ** median (range), *** number (%)

Among these 65 CMIA reactive women, 7 cases (10.8%) had RPR reactive and TPPA reactive results whereas 58 cases (89.2%) were RPR non-reactive. Among 58 women with RPR non-reactive results, 35 cases (60.3%) were TPPA non-reactive, which suggested that the initial CMIA results were false positive. As for the 23 cases with RPR non-reactive and TPPA reactive results, 7 cases had a history of past treatment for syphilis and 16 cases were considered as untreated, late, latent syphilis. No primary syphilis was identified in this study. A total of 23 cases needed syphilis treatment, which indicated that the prevalence rate was 1.98 per 1,000 pregnant women. If the traditional screening algorithm using RPR were used as the initial test, then 69.6% (16 in 23) of the cases with untreated syphilis would be missed.

The median CMIA S/CO value from all CMIA reactive women in this study was 2.86 with a range between 1.02 and 40.71 whereas the median CMIA S/CO value from cases with untreated syphilis was 21.27 with a range between 1.56 and 40.71. Since there were many high false positive results from the CMIA, we hypothesized that the S/CO at 1.00 may not be an appropriate cut-off point for pregnant women. Subsequently, we performed a sensitivity analysis by adjusting the S/CO cut-off point to decrease the false positive results of CMIA (Table 2). The S/CO cut-off point at 1.50 was able to decrease the false positive results from 60.3% to 37.8% without missing any untreated syphilis cases.

Discussion

Currently, EIA and CMIA for *T. pallidum* are increasingly used as the initial tests for syphilis screening because of their automation for screening large number of patients[5]. However, use in low prevalence populations may introduce problems of high false positive results[6,8] as seen in the United States Centers for Disease Control and Prevention (CDC) study which analyzed data from five laboratories where 140,176 specimens were screened for syphilis with the reverse sequence algorithm. 3.4% of the specimens were reactive EIA/CMIA whereas 56.7% had discordant results or non-reactive RPR[6]. When these discordant or non-reactive RPR samples were further tested with the confirmatory treponemal test with TPPA or FTA-ABS, it was discovered that 31.6% were false

Table 2. Results of the syphilis screening using CMIA with adjusted S/CO cut-off

S/CO cut-off	Total number of women	CMIA reactive		RPR non-reactive		TPPA non-reactive		Number of missing cases of untreated syphilis
		n	% of total	n	% of reactive CMIA	n	% of non-reactive RPR	
1.00	11,640	65	0.56	58	89.2	35	60.3	0
1.50	11,640	44	0.38	37	84.1	14	37.8	0
2.00	11,640	38	0.33	31	81.6	11	35.5	2
2.50	11,640	34	0.29	27	79.4	7	25.9	2
3.00	11,640	32	0.27	25	78.1	5	20.0	2

positive. These false positive results among discordant sera ranged from 25.2% to 60.0% in a low-prevalence population, which was higher than those from a high-prevalence population (12.2-18.6%). More recent reports have confirmed the high rate of false positive by CMIA[7-9].

Our study also confirmed that the use of CMIA in a low-prevalence setting could be problematic. The discordant results in the present study for reactive CMIA were as high as 89.2%. Furthermore, in the RPR non-reactive group, 60.3% had false positive results. The high proportions of discordant results and false positive results can be explained by our low prevalence of syphilis. Only 0.56% of all pregnant women in this study had reactive CMIA results that were much lower compared to the low-prevalence population from the CDC study, which had a range of reactive EIA/CMIA between 2.0-2.7%[6]. Two recent studies from China report a comparable reactive CMIA rate of 1.3 and 3.1%[7,8].

The prevalence rate of syphilis from the present study was 1.98 in 1,000 pregnant women. This figure is approximately 40% higher than the previous report[10] that used the traditional screening algorithm. The reason for this difference may be due to the study population. In the previous report, women from 9 provinces of Thailand including Bangkok were used. The women from the rural areas may exhibit different level of sexual activity compared to the women from the city of Bangkok. In addition, it is possible that the previous study used a different initial screening test compared to our study. For example, late, latent syphilis may result in false negative RPR or VDRL results[12]. As a result of this, the traditional screening algorithm using RPR or VDRL for the initial test can miss cases with late, latent syphilis. In the present study, initial screening use of CMIA was able to detect 16 cases with late, latent syphilis with non-reactive RPR. However, over-diagnosis should be cautioned. Because RPR can become negative after treatment, patients who cannot recall that they were previously diagnosed and treated for syphilis or have previously received antibiotics with activity against *T. pallidum* for other conditions may be misdiagnosed and over-treated. Due to the high false positive results of CMIA, clinicians should assess sexual risks, obtain clinical history including treatment received, and perform a complete physical examination before diagnosing syphilis. Cases with discordant results (reactive CMIA, but non-reactive RPR) should be retested with TPPA, the recommended confirmatory treponemal test [6,13].

Despite the high yield of false positive results, we found that when we adjusted the CMIA S/CO cut-off values, we were able to decrease the false positive results. This indicated that CMIA can still be used in a low-prevalence setting if the S/CO cut-off point was adjusted appropriately. We found that the S/CO cut-off point at 1.50 was able to decrease the false positive rate from 60.3% to 37.8% without missing any untreated cases. However, it is possible that this S/CO cuff-off point may not be appropriate for pregnant women from the rural areas where the prevalence of syphilis is much lower than in the city area. A side from that, we do not know whether the S/CO cut-off point should be different for the general population compared to those from the high risk groups such as the pregnant women and blood donors. Additional larger, multi-centered, prospective study is warranted to as certain the appropriate S/CO cut-off point that can be used across different geographic locations and socioeconomic status (SES).

One of the limitations of this study is due to its retrospective design. Even though there were standard checklists for sexual risk behaviors and history

of sexually transmitted diseases and treatment, yet for some patients, certain data were missing. This can be avoided in a larger, multi-centered, prospective study.

On the other hand, one of the strengths of this study is its large sample size from a single center. Furthermore, all of the results from the tests were performed by a validated, certified laboratory. This indicated that all of the results were precise.

In conclusion, syphilis screening using the CMIA as the initial test among pregnant women at KCMH had high discordant and false positive results, which can be rectified by increasing the S/CO cut-off point to 1.50. Hence, we recommend the use of reverse sequence algorithm to detect women with untreated syphilis but additional multi-centered, prospective study is necessary to determine the optimal S/CO cut-off point and cost-effectiveness of the syphilis screening among different geographic and SES pregnant women.

What is already known on this topic?

The use of CMIA for *T. pallidum* introduce problems of high false positive results, particularly in the low-prevalence population. These false positive results among discordant sera ranged from 25.2% to 60.0% in a low-prevalence population, which was higher than those from a high-prevalence population (12.2-18.6%).

What this study adds?

Our study reported the discordant results in Thai pregnant women for reactive CMIA were as high as 89.2%. However the initial screening use of CMIA was able to detect 16 cases with late, latent syphilis with non-reactive RPR. When we adjusted the CMIA S/CO cut-off values, we were able to decrease the false positive results. This indicated that CMIA can still be used in a low-prevalence setting if the S/CO cut-off point was adjusted appropriately.

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Potential conflicts of interest

None.

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ผลของการใช้ Chemiluminescent Microparticle Immunoassay ในการคัดกรองซิฟิลิสในหญิงตั้งครรภ์เอเชียที่มีความชุกต่ำและมีทรัพยากรจำกัด

อัมภิวัลย์ บุญช่วย, ปิยะ วงศ์จำปา, ณัฏฐิยา หิรัญกาญจน์, สุรสิทธิ์ ชัยทองวงศ์วัฒนา

วัตถุประสงค์: *เพื่อรายงานผลการใช้ chemiluminescent microparticle immunoassay (CMIA) ในการตรวจคัดกรองโรคซิฟิลิสในหญิงตั้งครรภ์ในเอเชีย*

วัสดุและวิธีการ: *ศึกษาผลการตรวจ CMIA ในหญิงตั้งครรภ์ที่คลินิกฝากครรภ์ โรงพยาบาลจุฬาลงกรณ์ โดยทำการศึกษาย้อนหลังจากการค้นข้อมูลหญิงที่มีผล CMIA เป็นบวกจากฐานข้อมูลของห้องปฏิบัติการและนำมาวิเคราะห์ต่อ ผลบวกสำหรับ Treponema pallidum กำหนดที่อัตราการดูดกลืนแสง ≥1.0 นำตัวอย่างที่ให้ผลบวกมาตรวจเพิ่มเติมด้วยวิธี rapid plasma reagin (RPR) และ Treponema pallidum particle agglutination assay (TPPA)*

ผลการศึกษา: *ผลการทดสอบที่รวบรวมระหว่างเดือนกุมภาพันธ์ พ.ศ. 2554 ถึงมกราคม พ.ศ. 2557 จากหญิงตั้งครรภ์ 11,640 คน พบมีหญิง 65 คน (0.56%) ที่มีผล CMIA เป็นบวกในกลุ่มนี้ 58 คน (89.2%) มีผล RPR เป็นลบ ในกลุ่มที่มี RPR เป็นลบ 35 คน (60.3%) มีผล TPAA เป็นลบและ 23 คน (39.7%) มีผล TPAA เป็นบวก จึงสามารถประมาณอัตราความชุกของโรคซิฟิลิสในประชากรกลุ่มนี้เป็น 1.98 ต่อหญิงตั้งครรภ์ 1,000 คน ในจำนวนนี้มีหญิงตั้งครรภ์ 7 คนที่เคยมีประวัติการรักษาซิฟิลิสบางส่วนและ 16 คนที่ไม่เคยรับการรักษาเลย ซึ่งอาจเป็นซิฟิลิสแฝงหรือซิฟิลิสช่วงปลายที่ต้องได้รับการรักษา ถ้าหากทำการคัดกรองด้วยการทดสอบ RPR หญิงตั้งครรภ์จำนวน 16 ใน 23 คน (69.6%) จะไม่ได้รับการรักษา*

สรุป: *แม้ว่า CMIA มีผลบวกปลอมสูงแต่การตรวจคัดกรองด้วยวิธีนี้ก็ถูกแนะนำให้นำมาใช้แทนขั้นตอนวิธีแบบดั้งเดิม อัตราผลบวกปลอมที่สูงอาจลดลงได้โดยการพิจารณาปรับค่า cut off ของอัตราการดูดกลืนแสงของ CMIA*