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Investigation of the rapid immunochromatographic test performance in the diagnosis of syphilis; comparison of four serological methods

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Abstract

Objectives: To test the performance of the newly available rapid test for syphilis, we compared it with Treponema pallidum hemagglutination assay (TPHA). Additionally, we investigated the performance of rapid plasma reagin (RPR) and chemiluminescence microparticle immunoassays (CMIA) at our laboratory using TPHA as a gold standard.

Methods: The serum samples of 595 patients with the pre-diagnosis of syphilis were studied by four serological methods. The sensitivity, specificity, and predictive values of RPR, CMIA, and syphilis rapid test were assessed by utilizing TPHA as a gold standard for the diagnosis of syphilis.

Results: Of the patients, 6.2% (37/595) had positive RPR, 5.5% (33/595) had positive CMIA, 5.5% (33/595) had a positive rapid immunochromatographic method and 5% (30/595) had positive TPHA. When TPHA results were taken as the reference, the sensitivity of the rapid test for syphilis was 100%, the specificity was 99.5%, PPV was 90.9%, and NPV was 100.0%.

Conclusions: It was observed that the rapid test for syphilis used in the study was quite successful, its cost was appropriate, and the test was very fast and easy to apply. At the same time, the agreement between syphilis rapid test and TPHA was found to be excellent.

Keywords: CMIA; rapid immunochromatographic test; RPR; syphilis serodiagnosis; TPHA.

Introduction

Syphilis represents a sexually transmitted disease (STD) that is caused by the spirochete Treponema pallidum which can be spread by sexual contact, by blood transfusion, and direct contact with lesions during primary and secondary periods [1]. In accordance with the World Health Organization (WHO) reports, about 17.7 million adults (15–49 years) around the world had syphilis in 2012, with estimated 5.6 million new cases each year [2]. Although the prevalence of syphilis varies widely between regions or countries, the highest prevalence rate is in Africa. More than 60% of new cases are diagnosed in underdeveloped countries [2]. Furthermore, it is estimated that more than 60% of cases of maternal syphilis worldwide are observed in Africa [3]. As to developed countries, especially in HIV-positive individuals, the incidence has been reported to have increased [1].

Serological methods are used in the diagnosis of syphilis due to the inability to produce the agent in culture. Non-treponemal tests, among the serological methods, used in the laboratory diagnosis of syphilis determine nonspecific antibodies against cardiolipins, while treponemal tests aim to identify specific antibodies that are formed against T. pallidum antigens. Venereal disease research laboratory (VDRL) and rapid plasma reagin (RPR) represent non-treponemal tests that are based on the reaction of cardiolipin with nonspecific antibodies generated in response to syphilitic infection [4]. However, these tests have low sensitivity and specificity depending on the pregnancy, autoimmune disorders, other infections and the periods of syphilis disease [5]. Thus, treponemal-specific tests such as enzyme immunoassay (EIA), T. pallidum hemagglutination assay (TPHA), microhemagglutination, fluorescent treponemal antibody absorption test (FTA-ABS), chemiluminescence microparticle immunoassays (CMIA), and the enzyme-linked immunosorbent assay (ELISA) detecting IgG antibodies to antigenic components of T. pallidum are utilized primarily for the purpose of confirming the diagnosis of syphilis in patients with a reactive non-treponemal test [6, 7].

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Nowadays, non-treponemal tests are used for screening in the conventional diagnostic algorithm of Syphilis, and positive results are verified by treponemal tests [8]. Treponemal tests are used for screening purposes in the reverse algorithm started to be used in the last decade or in the new diagnostic algorithm of Syphilis, and positive results are verified by a different treponemal test [9]. Treponemal tests are high specificity tests used for verification in the conventional algorithm and for both screening and verification purposes in the inverse/new algorithm. Their disadvantageous aspects are that they are not useful in the follow-up of treatment because they remain positive for years including the patients who have been treated. On the other hand, non-treponemal tests can be used for both screening and treatment follow-up [9].

In the present study, it was aimed to test the performance of the newly available rapid test for syphilis, we compared it with TPHA. Additionally, we investigated the performance of RPR and CMIA at our laboratory using TPHA as a gold standard.

Materials and methods

Study group

The serum samples of 595 patients with suspected syphilis that were sent to our laboratory in 2017–2018 (2-year period) were studied by four serological methods. Blood samples were collected from every participant and centrifuged until serum separation, and the actual laboratory tests were conducted. RPR, CMIA, syphilis rapid test, and TPHA were performed, and results were obtained in accordance with the kit’s manufacturer’s instructions. Characteristics of the methods in this study have shown in Table 1.

Rapid plasma reagin (RPR) test

Immuntrep RPR (Omega Diagnostics, UK) kit was used for this test. This test is a non-treponemal test for the detection of reagin/cardiolipin antibodies in human serum or plasma by flocculation. The principle of the test is based on the determination of aggregation in samples containing the antibody against the reagins at the end of the comparison of the patient sample and a reagent containing cholesterol/cardiolipin/lecithin. It takes 8 min to complete the test. The titrations of positive samples were obtained by serial dilutions at the ratios of 1/2 and its multiples to give semiquantitative results. The highest dilution ratio with positivity was reported in the final report.

Chemiluminescence microparticle immunoassay (CMIA)

Architect Syphilis TP (Abbott Japan Co, Japan) kit was used for this test. It is a treponemal test that detects specific antibodies that are formed against recombinant T. pallidum antigens in human serum or plasma with the help of chemiluminescence reaction. In the evaluation of the test results, the signal to cut-off value (s/co) ratio calculated by dividing the measurement values of the study samples and control serums by the cut-off value was taken as a basis. This ratio was considered to be negative between 0 and 1, and positive when higher than 1.0. It takes 26 min to complete the test. In the study, the results of the s/co values determined in the CMIA positive samples and the results of the TPHA and RPR tests were compared.

Rapid immunochromatographic assay

The syphilis rapid test (Hangzhou Biotest Biotech, People’s Republic of China) was used for this test. This is a treponemal test that detects IgG and IgM antibodies that are formed against T. pallidum by the immunochromatographic method. 75 μL of serum sample was used in this test, which could be studied using serum or plasma. The evaluation was made after 15 min.

Table 1: Characteristics of four serological methods in this study.

| Characteristics          | RPR          | Syphilis rapid test | Architect syphilis | TPHA          |
|-------------------------|--------------|---------------------|--------------------|---------------|
| Method                  | Agglutination| Immunochromatographic| Chemiluminescent microparticle immunoassay (CMIA) | Hemagglutination |
| Assay duration          | 8 min        | 15 min              | 26 min             | 60 min        |
| Specimen type           | Serum        | Serum or heparinized plasma | Serum              | Serum         |
| Sample volume           | 50 μL        | 75 μL               | 150 μL             | 10 μL         |
| Dilutions               | Manuel dilution| –                   | –                  | Manuel dilution|
| Device                  | Omega Diagnostics | Hangzhou Biotest   | Architect I 2000   | Omega Diagnostics |
**T. pallidum** hemagglutination assay (TPHA)

A commercial TPHA (Omega Diagnostics, UK) kit was used for this test. TPHA is a treponemal test based on the comparison of patient serums with sensitized erythrocytes in U-bottom microplate wells. Agglutination of sensitized erythrocytes is observed in the patient’s serum in the presence of specific *T. pallidum* antibody. The observation of agglutination was evaluated as positive in the 1/80 dilution of the patient serums, and the positive samples were re-studied in further dilutions, and the final titer was reported in the final report.

**Statistical analysis**

Number and percentage values were used to define the data. The sensitivity, specificity, and predictive values of RPR, CMIA, and syphilis rapid test in the present study were assessed by utilizing TPHA as a gold standard for the diagnosis of syphilis. The *kappa* value was calculated to assess the agreement between RPR, CMIA, syphilis rapid test, and TPHA. The *kappa* coefficient was interpreted as follows: 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; 0.81–0.99, excellent/almost perfect agreement. SPSS Statistics 21.0 program (IBM Corp, Armonk, NY, USA) was used in the analyses.

**Results**

Of the patients, 6.2% (37/595) had positive RPR, 5.5% (33/595) had positive CMIA, 5.5% (33/595) had a positive rapid immunochromatographic method and 5% (30/595) had positive TPHA. The number of patients who were positive in four methods at the same time was 28 (4.7%).

When TPHA was taken as the reference method, RPR in seven samples, CMIA in three samples, syphilis rapid test in three samples were found to be false positive. In the syphilis rapid test with RPR, false negative results were not observed, while in the CMIA, false negative results were obtained in two samples. It was observed that the s/co values detected in three samples identified as false positive by the CMIA method were below 12.

The sensitivity of the rapid immunochromatographic test for syphilis was 100%, the specificity was 99.5%, positive predictive value (PPV) was 90.9%, and negative predictive value (NPV) was 100.0%. The agreement between the TPHA and rapid immunochromatographic test was found to be nearly perfect with a *kappa* value of 0.99 (Table 2).

When TPHA results were taken as the reference, according to our study, the sensitivity of the RPR test method in the patient group was 100%, the specificity was 98.8%; the PPV was 81.1% and the NPV was 100.0% in the diagnosis of syphilis. The agreement between the TPHA and RPR tests was found to be nearly perfect with a *kappa* value of 0.93 (Table 3).

According to the TPHA results, the sensitivity of the CMIA test was 93.3%, the specificity was 99.1%, PPV was 84.8%, and NPV was 99.6%. The agreement between the TPHA and CMIA tests was found to be nearly perfect with a *kappa* value of 0.98 (Table 4).

**Discussion**

The tests and their order to be selected for the screening and confirmation in the diagnosis of syphilis are important for accurate diagnosis. The conventional algorithm gives more reliable results in populations with high prevalence. Furthermore, the activity of the infection can be determined by this algorithm [10]. The sensitivity and specificity in the conventional algorithm are low, and the false

| Syphilis rapid test | Negative | Positive | Total |
|---------------------|----------|----------|-------|
| TPHA                |          |          |       |

Sensitivity of syphilis rapid test (30 of 30 samples), 100%; specificity of syphilis rapid test (562 of 565 samples), 99.5%; agreement (30 + 562=592, of 595 samples), 93.7%; positive predictive value (30/37) 81.1%; and negative predictive value (558/558) 100%.

| RPR     | Negative | Positive | Total |
|---------|----------|----------|-------|
| TPHA    |          |          |       |

Sensitivity of RPR (30 of 30 samples), 100%; specificity of RPR (558 of 595 samples), 98.8%; agreement (30 + 558=588, of 595 samples), 93.7%; positive predictive value (30/37) 81.1%; and negative predictive value (558/558) 100%.
In various studies, the sensitivity and specificity of RPR were comparable to the findings reported in our study [19–21]. The RPR specificity in our study was determined to be 98.8%. Similar results were obtained in different studies (96.9 and 100%) [22, 23]. The difference in the high specificity values may be due to reasons such as commercial kit, subjective evaluation, personnel experience. For example, the reduction of the margin of human error due to the preference of the automated system in the mentioned studies may have increased the specificity. In our study, the RPR positive and TPHA negative results of the 9 (1.7%) patients obtained by the conventional algorithm were evaluated as false positive reactions.

Another treponemal test used in the laboratory diagnosis of syphilis is CMIA. This test is studied using automated chemiluminescence devices. In this way, a large number of serum samples can be studied at the same time, and the results are evaluated objectively [24, 25]. Therefore, the use of CMIA is appropriate for centers with a high number of patients [24]. Studies with similar sensitivity and specificity (90–100%) were found in the literature [26–30]. However, some studies reported different values from our study [31–33]. This change in the performance of the CMIA test is due to the type of immunodominant syphilis proteins. The PPV and NPV values of the CMIA test were determined as 84.8 and 98.8%, respectively. Similar PPV and NPV values were also reported in various studies [26, 28, 32].

In our study, the CMIA results of 5 (0.8%) patients evaluated as CMIA positive and TPHA negative were determined to be false positive. Moreover, the presence of a relationship between the TPHA results and CMIA values was also investigated, and CMIA positive cases below 12 s/co were found to be TPHA negative. Although this result was not verified by another method, which is accepted as a gold standard such as FTA-ABS, it suggests that the CMIA test may give false positive results at low s/co values. Therefore, CMIA results were suggested to be repeated one month later in accordance with the diagnosis algorithm for CMIA positive and TPHA negative samples. There are also studies in the literature indicating that the CMIA test can give false positive results at low s/co values. Young et al. reported that the s/co values of the samples which were stated as false positives were ≤5 [34]. Similarly to our study, Ozbek et al. reported that s/co values give false positive results when they are <12 [35]. According to the data we obtained, low s/co values have a risk of false positivity. However, in accordance with the guidelines published in recent years, it would be appropriate to use the treponemal tests including the CMIA and the rapid immunochromatographic test for syphilis in screening. In case of a

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**Table 4:** Comparison of TPHA and chemiluminescence microparticle immunoassays (CMIA) test results.

|            | CMIA Negative | CMIA Positive | Total |
|------------|---------------|---------------|-------|
| TPHA Negative | 560           | 2             | 562   |
| TPHA Positive | 5            | 28            | 33    |
| Total       | 565           | 30            | 595   |

Sensitivity of CMIA (28 of 30 samples), 93.3%; specificity of CMIA (560 of 565 samples), 99.1%; agreement (28 + 560 + 588, of 595 samples), 98.8%; positive predictive value (28/33) 84.8%; and negative predictive value (560/562) 99.6%.

Positivities are determined to be high. Therefore, the results need to be verified [11]. In the inverse/new syphilis diagnosis algorithm, treponemal tests are used as a screening test unlike the conventional algorithm [10]. The results determined as positive are verified by a second and different treponemal test. The inverse algorithm is a cost-effective approach in laboratories with a high number of samples, and it gives more reliable results in populations with low syphilis prevalence [12].

Among the non-treponemal tests, the RPR test is often preferred because of its ease of application, rapidity, and cheapness. However, as the cardiolipin antigens used in these tests are not specific, the fact that false positives can be observed in autoimmune patients, pregnant women, and drug addicts is the disadvantage [13, 14]. Among the treponemal tests, the TPHA test is another advantageous test because of its low cost and ease of application. Subjective evaluation and non-automation of the test are its disadvantages [15].

A novel treponemal test group that has started to be used recently and attracted global attention is rapid immunochromatographic tests for syphilis. Among these tests based on the qualitative lateral flow immunochromatographic method, Syphilis Health Check kit is the only test with US Food and Drug Administration (FDA) approval (Trinity Biotech USA, Inc., NY). Differently from conventional laboratory-based syphilis treponemal tests, the TPHA test is another advantageous test because of its ease and application. Subjective evaluation and non-automation of the test are its disadvantages [15].

In our study, the CMIA results of 5 (0.8%) patients evaluated as CMIA positive and TPHA negative were determined to be false positive. Moreover, the presence of a relationship between the TPHA results and CMIA values was also investigated, and CMIA positive cases below 12 s/co were found to be TPHA negative. Although this result was not verified by another method, which is accepted as a gold standard such as FTA-ABS, it suggests that the CMIA test may give false positive results at low s/co values. Therefore, CMIA results were suggested to be repeated one month later in accordance with the diagnosis algorithm for CMIA positive and TPHA negative samples. There are also studies in the literature indicating that the CMIA test can give false positive results at low s/co values. Young et al. reported that the s/co values of the samples which were stated as false positives were ≤5 [34]. Similarly to our study, Ozbek et al. reported that s/co values give false positive results when they are <12 [35]. According to the data we obtained, low s/co values have a risk of false positivity. However, in accordance with the guidelines published in recent years, it would be appropriate to use the treponemal tests including the CMIA and the rapid immunochromatographic test for syphilis in screening. In case of a
positivity was detected in screening, a verification should be performed with treponemal tests such as TPHA and FTA-ABS. Also, RPR can be studied to contribute to the evaluation of the patient in terms of active syphilis and to make a possible treatment follow-up.

Unfortunately, our study had a limited number of syphilitic patients because of the low prevalence of syphilis in our country, so the number of samples was small and could not been classified according to syphilis stage. Furthermore, samples evaluated as TPHA positive were not sent to the reference laboratory for verification by the FTA-ABS test.

In conclusion, it was observed that the rapid immunochromatographic test for syphilis (sensitivity, specificity, and predictive values) used in the study was quite successful, its cost was appropriate, and the test was very fast and easy to apply. It can be used for screening and diagnosis. At the same time, the agreement between syphilis rapid immunochromatographic test and TPHA was found to be excellent.

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