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EVALUATION OF THREE RAPID SEROLOGICAL TESTS AS SCREENING TESTS FOR DIAGNOSIS OF SYPHILIS

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ABSTRACT

Serology plays an important role in diagnosis of syphilis. Over a past few decades there has been renewed interest in the subject due to rise in the prevalence of Human Immunodeficiency Virus (HIV) and syphilis together. The diagnosis of syphilis may be more complicated in patients with HIV because of atypical clinical presentation and also due to false-negative and false-positive serologic results.

In present study we evaluated the utility of 3 rapid tests as screening tests as against standard VDRL and specific TPHA tests. A total of 172 serum samples were subjected to VDRL, TPHA, Spirolipin, SYPH C, and Signal TP (span diagnostics Ltd) tests and grouped into two groups according to their HIV status. 43 sera were HIV reactive and 129 were HIV non reactive.

All these tests have overall good sensitivity (spiroliptn-2-72.5%,Spirolipin 1-82.22%,Syph C-86.67%,Signal TP-82.22%) and specificity but their sensitivity was very low in HIV positive patients(Spirolipin 2-50%,Spirolipin 1-57.89%,Syph C-73.68%,Signal TP-68.42%).

So these tests can be used as rapid screening tests in field area for diagnosis of syphilis but limits its use in HIV positive patients because of low sensitivity.

Introduction-

Syphilis is an ancient disease. The causative agent Treponema pallidum is very fragile and delicate. Its detection is possible only in lesions in primary syphilis. Obviously serology plays an important role in diagnosis. Over a past few decades there has been renewed interest in the subject due to rise in the prevalence of Human Immunodeficiency Virus (HIV) and syphilis together(1). Syphilis as well as other genital ulcer diseases may increase the risk of the acquisition and transmission of HIV. The diagnosis of syphilis may be more complicated in patients with HIV because of atypical clinical presentation and also due to false-negative and false-positive serologic results (2).

Nontreponemal serologic tests for syphilis measure antibodies against cardiolipin- antigens. Veneral Disease Research Laboratory (VDRL) test is a conventional test done in many laboratories for diagnosis of syphilis. But it is time consuming and demands expertise. In addition, results are affected by personal bias.

TPHA is a very specific and gold standard test however it needs technical expertise.

In the present study we have compared three rapid serological tests(Signal TP,Spirolipin and Syph C) with the standard VDRL test and the specific TPHA test.

Material and methods- A total of 172 serum samples received in serology lab for VDRL were included in study and subjected to VDRL and TPHA tests both. The same sera were tested by 3 rapid tests viz. Spirolipin, SYPH C, and Signal TP (Span diagnostics LTD).A written consent was obtained from all these patients for HIV testing and the sera were tested by Combaids test. Positive sera in first test were tested by other two tests with different principles (Tri-dot and Parikshak test).These sera were grouped into two groups according to their HIV status viz HIV positive and HIV negative.

VDRL test- For VDRL test, sera were inactivated in waterbath at 560c for half an hour. VDRL antigen (obtained from serologists Calcutta) was freshly prepared. The test was performed as per manufacturer's instructions. The results were read as-

No clumps/very slight ruffness - Non reactive
Small clumps - Weakly reactive
Medium/large clumps - Reactive

Quantitative tests were performed on all reactive and weakly reactive sera .Titers of 1:8 and above were considered as significant titres.

Treponema Pallidum Haemagglutination test (TPHA)-

TPHA test was performed by using a commercial kit (Randox Laboratories LTD).

Procedure-Qualitative test- In a microtitre plate 25 microL of 1:20 diluted serum sample was taken in test well and control well. 75 MicroL of control cells (non sensitized suspension of stabilized avian cells) were added to control well. 75 MicroL of test cells (stabilized suspension of avian cells sensitized with Nichol's Treponema pallidum antigen) were added to test well. The microtitre plate was incubated in dark at room temperature for 45-60 min.

The results were interpreted as follows-

Degree of haemagglutination	Grade	Interpretation
Uniform mat of cells covering entire base of well sometimes with folded edges	4+	Reactive
Uniform mat of cells partially covering base of well	3+	Reactive
Smaller mat surrounded by a ring of cells	2+	Reactive
Smaller mat surrounded by a smaller more distinct ring of cells	1+	Reactive
Well defined dense ring with a hole in centre	+/-	Indeterminate
Definite button of non agglutinated cells sometimes with a small hole in centre	Negati ve	Non reactive

Signal TP test-

Signal TP Ver 2.0 flow through is an antitreponemal spot/Immunodot test in which purified recombinant treponemal antigens are immobilized onto a porous nitrocellulose membrane of the filtration device, as a capture agent. As the sample flows

through the membrane, the immobilized antigen on the membrane traps antitreponemal antibodies. This is revealed by magenta red coloured dot/spot.

Spirolipin test-
In this flow through test the major immunoreactive antigens (47KDa, 17 KDa) are immobilized in a single dot on a flow through platform. On the same platform two more dots, one of VDRL antigen and second of control antigen were also immobilized.

As the serum sample flows through the membrane, the immobilized antigens trap the antibodies (antitreponemal/reagin). The presence of specifically bound antibodies is indicated by a red-magenta coloured dot/spot. The dot of Spirolipin 2 indicates nonspecific reagin antibodies while that of spirolipin 1indicates specific antitreponemal antibodies.

Syph C-
The Syph-C test for syphilis employs purified recombinant antigen/s (47 KDa & 17 KDa....) that are found to be major immunoreactive antigens are spotted on the teeth of a plastic comb. When the comb is incubated in diluted serum (1:3), the specific antibodies if present, bind to the antigens and it is indicated by a magenta red coloured spot.

All tests were performed and evaluated according to the manufacturers instructions.

RESULTS-Total 172 serum samples were included in study out of which 43 were HIV positive and 129 were HIV negative.

The results of VDRL, TPHA and the three rapid tests (mentioned above) in both these groups were as follows (table no 1)

Table no-1 –Results of tests in HIV positive and negative groups

	HIV positive group (n=43)	HIV negative group (n=129)
VDRL reactive	18	22
TPHA positive	19	26
Spirolipin(2)	9	26
Spirolipin(1)	13	29
Syph c	14	27
Signal TP	15	28

Spirolipin 2, which is a nonspecific treponemal spot test, was compared with the nonspecific VDRL test. Spirolipin 1, Syph C, Signal TP (all specific treponemal tests) were compared with TPHA. The sensitivity, specificity, positive predictive value (PPV) and Negative predictive value (NPV) were calculated by using statistical methods.

Table no 2- Sensitivity and specificity ot rapid tests in percentage (n=172)

Test	Sensitivity	Specificity	PPV	NPV
Spirolipin (2)	72.5	95.45	82.85	89.36
Spirolipin (1)	82.22	96.06	88.09	93.84
Syph C	86.67	98.43	95.12	95.41
Signal TP	82.22	95.28	86.04	93.79

Presuming the sensitivity and specificity of VDRL and TPHA as 100%, all these tests performed well.

Table no 3- Sensitivity and specificity in HIV reactive and Non-reactive group

	HIV positive group (n=43)				HIV Negative group (n=129)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Spirolipin(2)	50%	100	100	73.52	90.91	94.39	76.92	98.05
Spirolipin(1)	57.89	91.67	84.61	73.33	100	97.09	89.65	100
Syph C	73.68	100	82.75	73.68	96.15	98.06	92.69	99.01
Signal TP	68.42	91.67	88.66	68.42	92.31	96.12	85.71	98.01

In HIV negative patients, these tests have good sensitivity and specificity however in HIV positive patients, their sensitivity decreased without much affecting the specificity.

Discussion-
Considering the results, it can be said that all these three rapid tests have good sensitivity and specificity. But when we compared the two groups of HIV positive and HIV negative patients, we found that these tests showed good specificity even in HIV positive group. The sensitivity of these tests decreased in HIV positive patients.

In HIV negative patients, spirolipin 2 test has good sensitivity and specificity (90.91% and 94.39% respectively) however out of 43 HIV positive patients, VDRL was reactive in 18 patients but spirolipin 2 was positive in 9 patients only.(sensitivity 50%)

Comparing the results of Spirolipin 1(specific test) with TPHA, it can be seen that the sensitivities and specificities are comparable in HIV negative group;however in HIV positive patients,the negative predictive value of spirolipin 1 reduced considerably maintaining its specificity.

In HIV negative group, the other two tests Syph C and signal TP, also showed good sensitivity (96.15%and 92.31% respectively)and specificity(98.06 and 96.12% respectively) as compared to TPHA. In HIV positive group however sensitivities of the both tests decreased considerably (73.68 and 68.42% respectively). Overall suppression of immune system leading to poor antibody response in HIV positive individuals could be the reason for this phenomenon (3). Though we have not taken into consideration CD4 counts of all these patients, there is definitely low immune response in patients with HIV infection.

Ross et al has also mentioned the same phenomenon that antibody tests for syphilis may be unreliable in patients co-infected with HIV because of suppressed immune system (4). According to Synthes et al treponemal and non-treponemal tests are undersensitive in persons at risk for repeated exposure to T. pallidum (5).

According to Hall et al, serologic tests appear to be accurate and reliable though unusal serological responses have been reported in HIV infected patients with syphilis. The specificity of non-treponemal tests may be compromised in HIV infected persons. The high chances of Biological false positive reactions in VDRL test, in HIV positive population has been reported (2). In our study, we found that in HIV negative patients the results of VDRL, TPHA and the three rapid tests mentioned above were comparable.In HIV positive group, the difference in the seropositivity exhibited by these tests was evident In the present study, we have studied the performance of VDRL, TPHA and the other three rapid tests in HIV positive and HIV negative patients with syphilis.In the next phase of this study we are planning to study and compare the performances of these tests with the most commonly used (and FDA approved) Rapid Plasma Reagin (RPR) test and VDRL ELISA test.

CONCLUSION-
Spirolipin,SYPH C,and Signal TP can be used as rapid screening tests in the field for diagnosis of syphilis but with some limitations for their use in HIV positive patients.

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