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COMPARATIVE STUDY OF RAPID PLASMA REAGIN TEST WITH ELISA IN THE SERODIAGNOSIS OF SYPHILIS.

Microbiology

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ABSTRACT

Syphilis is a venereal disease, transmitted by Treponema pallidum. Symptoms occur in three stages.

Aims and objective:

To evaluate diagnostic performance of ELISA in the detection of anti- Treponemal antibodies and to compare the sensitivity and specificity of ELISA with RPR & in diagnosing latent and tertiary syphilis.

Materials and methods:

It's a cross sectional study conducted for one year in a tertiary care hospital. 180 Blood samples were received in our lab from the patients who were suspected to have symptoms of syphilis to do RPR, ELISA and TPHA.

Results& Discussions:

With male preponderance, 25-34 years age group have high percentage of syphilis. Out of the total 180 serum samples ELISA (45%) shows high reactivity when compared with TPHA & RPR.

Conclusion: Accurate diagnosis of syphilis in antenatal mothers helps in preventing progress of the disease and also the onset of congenital syphilis.

KEYWORDS

Syphilis, RPR, ELISA, TPHA.

INTRODUCTION:

Venereal syphilis, is a very ancient disease. Syphilus, is the name of a young Shepard in a poem written by, GirolamoFracastoro.[1] WHO estimates the incidence of syphilis in 15-49 years is 5.6 million. Globally the incidence of Syphilis was 10.6 million cases in 2008.[2]

Increase in the incidence of syphilis correlates well with the increased transmission of HIV -1 infection.[3] Biological false positives and false negatives can occur with RPR test, in patients with co-infection.[4]

Pregnancy itself gives BFP reaction in RPR. So a pregnant women should undergo a sensitive screening test to diagnosis and treat syphilis, to decrease the burden of congenital syphilis.

The congenital syphilis diagnosis are difficult .Passively transferred maternal antibodies will give reactive RPR for 6 months and positive treponemal tests for 12 to 18 months in the infants. Demonstration of IgM antibodies along with positive treponemal test is essential to diagnosis congenital syphilis.[5]

The symptoms of primary, secondary and tertiary syphilis may mimic number of diseases, which requires a reliable serological test to arrive at a diagnosis.And also the asymptomatic latent stage requires the same.

A sensitive alternate screening diagnostic tool is needed to provide proper treatment to the patient. Though RPR is useful in follow up of syphilitic patient after treatment, its sensitivity is low in early, latent, tertiary and stages of syphilis.

WHO gave the guidelines to use Treponemal antigen based enzyme Immuno assay as an alternative screening test for the diagnosis of syphilis.[6] In this study, ELISA is used as an alternate screening test with RPR. The sensitivity and specificity of both the screening tests are compared with the Gold standard, "Treponema pallidum Hemagglutination test"

MATERIALS AND METHODS:

This comparative study of RPR and ELISA is a cross sectional study. The study had been accepted by the ethics committee.

The study group included are the patients of all age groups and both sexes who have clinically suspected history and symptoms of syphilis and history of high risk sexual behavior. Size of the study group is 180 patients.

After getting consent and history from the patient, 3ml of blood was collected without anticoagulant. RPR test was done with the separated serum.

All the reactive serum samples in RPR and the non-reactive samples, with history suggestive of syphilis were subjected for ELISA & TPHA.

Rapid plasma reagin Qualitative test Procedure:

40 µl of serum was added to the white colored slide, marked with circles. Same amount of negative and positive controls were added separately to the wells. 18 µl of RPR reagent was added to each circle, mixed well and the slide was rotated gently for 8 minutes to look for flocculation

Samples are interpreted as reactive when fine ,medium & large aggregates of carbon particles are seen with button formation as non-reactive. Further titre for the reactive samples are seen by semi quantitative method after diluting the sample with physiological saline.

Enzyme linked Immuno Sorbent assay:

A qualitative sandwich ELISA detecting IgM, IgG and IgA antibodies in the serum specific for recombinant Treponemal antigens (47Kd, 17Kd) was performed in this study .

The absorbance value of negative control should be < 0.1 & for positive control > 1.0.

Cut off value is calculated by adding average of negative control absorbance with 0.2.

Absorbance value of samples with more than cut off value is interpreted as positive & less than it as negative.

A qualitative Treponema pallidum Haemagglutination test was done by using patient's sample, sample diluent, negative control (serum pre diluted in buffer without antibodies), positive control (serum pre diluted in buffer containing antibodies) test cells(formolised tanned Fowl red cells sensitized with Treponema pallidum antigen) Smooth mat covering the entire bottom of the well or with thin or thick red circle at the periphery of the mat were interpreted as reactive sample for treponema pallidum specific antibodies. Compact button of red cells with and without a central hole as negative reaction.

RESULTS & DISCUSSIONS:

During this one year study period 180 blood samples were collected.

These serum samples were subjected for RPR for anti cardiolipin antibodies, ELISA for testing antibodies to recombinant Treponemal antigen and TPHA. The results of ELISA and RPR were compared with that of TPHA, which was a specific test for Syphilis.

The statistical analysis was done by Statistical Package for Social Sciences (SPSS) version 16.0 software. The statistical significance (P <0.05) were performed by McNemar's and Fisher's chi square test

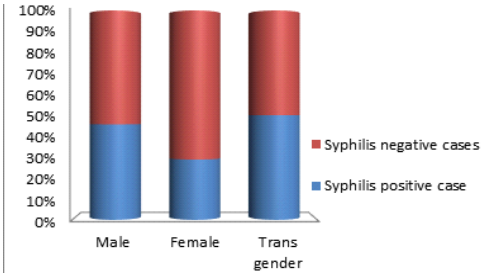
Sensitivity, Specificity, Positive Predictive value (PPV) and Negative Predictive Value (NPV) were calculated and given with their estimate.

The age of the patient under study ranged from 15 - 65 years. Majority of them (31.7%) belong to 25 – 34 years of age with male predominance 103 (57.2%) followed by females 73 (40.56%) and then by trans genders 4 (2.22%)

The highest percentage (30.0%) of Syphilis positive patients belong to 25-34 years of age with high Syphilis positive cases in males (67.1%), followed by females (30.00%) and then by trans genders (2.90%)

But the study shows higher prevalence of Syphilis (50.0%) in trans gender due to the perverted sexual practices prevalent among them.

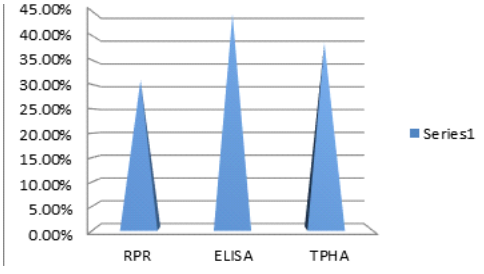
1. sex wise distribution of Syphilis in study population



Higher prevalence of Syphilis in trans gender.

Among the study samples , 55 (30.5%) of them were reactive by RPR test, 81 samples (45%) positive by ELISA and 70 samples (38.9%) positive by TPHA test.

2. Distribution of Syphilis by serological tests



ELISA shows high reactivity

Among the Syphilis positive cases, 14 secondary Syphilis, 55 latent Syphilis, 1 tertiary Syphilis cases without symptomatic primary syphilis were seen. This could be due to the usage of common antibiotics like penicillin, Azithromycin, Doxycycline used in treating Syphilis are also used in other medical conditions commonly in our society. The number of latent Syphilis cases were more in this study.

The overall reactivity for ELISA (45%) was higher in this study when compared to the other two tests and the results are similar in the following studies done by Narinder Kaurnaidu et al[7] & Rita Castro et al[8].

When RPR was compared with TPHA the sensitivity, specificity, PPV and NPV were found to be 60%, 88%, 76.4% and 77.6% respectively .The P value was 0.019.

Table 1:Cross tabulation of RPR results with TPHA (n=180)

RPR	TPHA		Total
	Positive	Negative	
Positive	42	13	55
Negative	28	97	125
Total	70	110	180

Among 55cases , 42 were positive and 13 were negative by TPHA. In this 42 TPHA positive samples, 11 were reactive with ≥ 1:16 dilution and remaining 31 samples show low dilution reactivity of ≤ 1:8. But ELISA shows positivity for 40 of these samples. This low sensitivity of RPR is supported by Saral et al[9] study.

Similarly when ELISA was compared with TPHA the sensitivity, specificity, PPV and NPV were 90%, 83.6%, 77.8% and 93% which shows that ELISA is more sensitive (90%) than RPR (60%) in syphilis diagnosis. P- value obtained is 0.028. But its specificity (83.6%) is lower than RPR (88%). This is compared with a study by Woznicova V et al[10].

Table 2: Cross tabulation of ELISA results with TPHA (n=180)

ELISA	TPHA		Total
	Positive	Negative	
Positive	63	18	81
Negative	7	92	99
Total	70	110	180

63 cases were positive & 18 were negative by TPHA among the 81 ELISA positive cases.When RPR & ELISA was compared with TPHA, their sensitivity and PPV in secondary syphilis was 85.71% ,100% and 100%,100% respectively. In the total 14 cases,12 were reactive & 2 non-reactive in undiluted serum due to prozone phenomenon (1:128& 1:64 dilutions) in RPR which indicates that sensitivity of ELISA (100%) is higher than RPR (85.71%).

Also RPR and ELISA shows sensitivity & PPV of 50.90% ,100% and 87.3% and 100% respectively in diagnosis of latent syphilis when compared with TPHA showing the low sensitivity of RPR in this stage. This is supported by a study done by Dzintarsozolinset al.[11]

In this study, there were 27 non-reactive samples by RPR in latent Syphilis when compared with TPHA. Among these ELISA showed positivity for 20 samples except for 7 samples. Also 4 of samples from ante natal mother which was TPHA positive were reactive in low titre in RPR misleading as biological false positive reactions.

Table3: Evaluation of RPR with TPHA in latent syphilis (n=55)

RPR	TPHA		Total
	Positive	Negative	
Positive	28	0	28
Negative	27	0	27
Total	55	0	55

A case suspected to have tertiary syphilis of ophthalmic involvement whose serum was screened by all the 3 tests shows RPR of 1:4 dilution reactive & 100% sensitivity and PPV by ELISA with that of TPHA. This low titre gives a false impression of biological false positive reactions again.

CONCLUSION: It is evident that, ELISA have good sensitivity and PPV when compared to RPR in the screening and diagnosis for Syphilis. The low titre of RPR in latent Syphilis and in antenatal mothers should be followed by ELISA and a specific test. Wrong interpretation as false positive or false negative will show ill- effect on the patient.

Once diagnosed as Syphilis, RPR can be used for follow up of patients to see their response to treatment and for staging of Syphilis. Using single serological test in the diagnosis of Syphilis should be avoided due to the limitations and benefits of each tests.

In this study, it is concluded that all serum samples subjected for screening and diagnosis of Syphilis must undergo a better screening test like ELISA and confirmed by a specific test like TPHA to alleviate the false positives.

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