



A STUDY ON THE SERODIAGNOSIS OF SYPHILIS BY COMBINATION OF TPHA & RPR IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Aim & Objectives: The present study aims to determine the role of the Treponema Pallidum Hemagglutination (TPHA) test in RPR reactive and non-reactive sera for serodiagnosis of syphilis and also objectifies to assess the Biological false positives. **Materials and Methods:** It is a cross-sectional study was conducted in the Department of Microbiology, in association with ANC, DVL, and ART clinics GGH, Guntur, from January 2022 to June 2022. 300 Blood samples received from attendees OPDs of ANC, ART, and DVL (100 samples from each group) were subjected to RPR (non-treponemal test) and TPHA test for detection of specific anti-treponemal antibodies and assessment of Biological false positives. Ethical approval was taken before the initiation of the study. **Results:** Mean age in ANC was 26yrs, in ART was 31 yrs, in DVL was 30 yrs, and male to female ratio was 1.7:1. Out of 300 samples, 19 (6.33%) were qualitatively reactive for RPR, and 18(6%) were TPHA Positive. Among 19 RPR reactive, ART was 8 (42%), DVL was 8 (42%), ANC was 3 (16%) samples. 18 TPHA positives, DVL was 9 (50%), ART was 7 (38.88%), ANC was 2 (11.11.%) samples. Both RPR Reactive and TPHA Positive were 15, in that DVL was 8 (53.33%), ART was 6 (40%), ANC was 1 (6.6%) sample, suggestive of True positives. RPR Reactive & TPHA Negative were 4, in that ANC was 2 (50%), ART was 2 (50%) suggestive of biological false positives but not found in DVL. RPR non-reactive & TPHA Positive were 3, in that ANC was 1 (33.33%), ART was 1 (33.33%), DVL was 1 (33.33%), suggestive of past infection with complete treatment. **Conclusions:** Specific tests to diagnose syphilis like TPHA in combination with non-specific test RPR will differentiate the true positives from BFP's. Positive TPHA indicates past/present infection. RPR reactivity in high dilutions will indicate the activity of the disease and has got a prognostic value of the treatment. My study concludes that the TPHA test should be used for routine confirmation of a positive RPR test irrespective of its titer.

KEYWORDS : Syphilis, TPHA, RPR

INTRODUCTION

Syphilis is a sexually transmitted disease (STD) caused by Treponema pallidum (T. pallidum) Presents with different stages and a prolonged course, Undiagnosed and untreated syphilis can damage major organs like the heart and brain, among antenatal mothers it causes stillbirth and spontaneous abortion (40%), perinatal death (20%).¹

Despite the availability of relatively effective and affordable treatment, an estimated 7 million new syphilis infections occur globally. [Peeling and Hook, 2005, WHO 2020].^{2,3}

According to IUSTI guidelines, serological tests for syphilis remain the diagnostic standard and at least one treponemal and one non-treponemal test should perform to rule out BFPs.^{4,5,6}

Therefore, the present study is taken up to evaluate the serodiagnosis of syphilis by the combination of TPHA & RPR

MATERIALS AND METHODS

Study Design, Period:

- A cross-sectional study was conducted in the Department of Microbiology, in association with ANC, DVL, and ART clinics GGH, Guntur, from January 2022 to June 2022.

Study Participants:

- A total of 300 participants were included in this study with their consent, 100 attendees from each DVL, ANC, and ART clinic.
- Detailed History of all patients was taken. Specimen Collection and Processing:
- Serum was separated from a blood sample collected from each participant, centrifuged, and Processed for qualitative RPR & TPHA
- RPR reactive samples were quantitatively titrated.
- A Reactive sample is indicated by macroscopically visible black

flocculation of carbon particles, whereas non-reactive samples appear as smooth and uniform.

- Results were recorded as Reactive and non-reactive
- Positive & negative controls were included.
- The TPHA test was performed qualitatively for all samples, and quantitatively for positive samples, Positive Control & Negative Control included.
- Test interpreted as Positive if it shows agglutination of a cell at the bottom of the microtitre plate.
- Without agglutination, a compact button was taken as TPHA Negative.

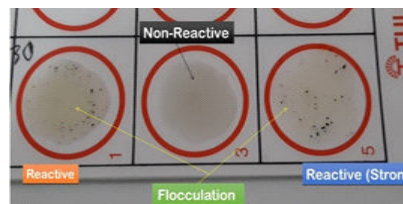


Figure 1

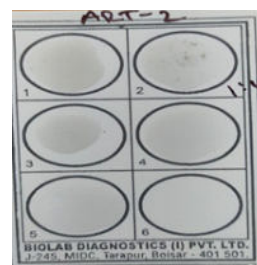


Figure 2

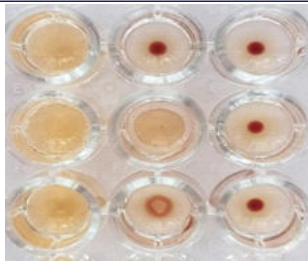


Figure 3

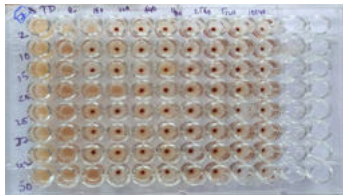


Figure 4

RESULTS

Out of 300 samples, n=19 (6.33%) was qualitatively reactive for RPR, and n=18 (6%) was TPFA Positive. The mean age in ANC was 26yrs, in ART was 31 yrs, and in DVL was 30 yrs. Male to female ratio noted is 1.7:1. (Figure 5)

Out of 100 ANC samples, 3 were RPR REACTIVE and 2 were TPFA POSITIVE.

Out of 100 ART samples, 6 males were both RPR REACTIVE and TPFA POSITIVE, and out of 3 females, 2 were RPR REACTIVE, and 1 was TPFA Positive.

Out of 100 DVL samples, 6 males were RPR REACTIVE and 7 were TPFA POSITIVE and 2 females were both RPR REACTIVE and TPFA POSITIVE, respectively. (Table 1)

The mean age in ANC was 26yrs, in ART was 31 yrs, and in DVL was 30 yrs. Male to female ratio is 1.7:1. (Table 2)

The low titer values indicate early stages of primary syphilis or maybe a better-responding case to the treatment who has H/o syphilis or may be BFP (biological false positives.) (Table 3)

The distribution of RPR TPFA among the study groups was tested. True positives were 15 in number. (Table 4)

A comparison of the present study results was done in Table 5.

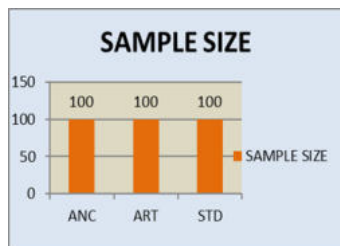


Figure 5 Sample size

TABLE 1: GENDER-WISE DISTRIBUTION OF RPR & TPFA REACTIVE CASES

STUDY GROUP	ANC	ART		DVL	
		MALE	FEMALE	MALE	FEMALE
RPR	3	6	2	6	2
TPFA	2	6	1	7	2

TABLE 2: AGE WISE DISTRIBUTION OF EACH STUDY GROUP

STUDY GROUP	ANC	ART		DVL	
		male	female	male	female
<20 yrs	32	2	0	10	0
21-30	64	36	16	44	8

31-40	4	28	14	28	6
41-50	0	4	0	2	2
>50 yrs	0	0	0	0	0
TOTAL	100	100	100	100	100

TABLE 3: DISTRIBUTION OF ANC, DVL, AND ART PATIENTS IN DIFFERENT QUANTITATIVE TITERS OF RPR

Reactive VDRL titer	ANC	DVL	ART
1:2	0	1	0
1:4	1	3	4
1:8	0	3	2
1:16	0	0	0
1:32	0	0	0
NO TITRE	2	1	2
TOTAL	3	8	8

TABLE 4: DISTRIBUTION OF RPR TPFA AMONG THE STUDYGROUPS

STUDY GROUP	Total Samples tested	RPR REACTIVE	TPFA POSITIVE	RPR + TPFA +	RPR + TPFA -	RPR - TPFA +
ANC	100	3	2	1	2	1
ART	100	8	7	6	2	1
DVL	100	8	9	8	0	1
TOTAL	300	19	18	15	4	3
				TRUE POSITIVES	BFP	TREATED CASES

TABLE 5: COMPARISON WITH OTHER STUDIES

Variables	Sivakumar S. Banupriya K.	Arti B. Ninama, et al	Manju Bala, et al	Markos Negash et al	Present Study
Age	-	30-40yr (39%)	20-29yr (38%)	22-32yr (77%)	21-30yr (38%)
Gender	59%M 41%F	56%M 44%F	38%M 62%F	52%M 48%F	63%M 37%F
RPR +	17%	12%	11.4%	50%	6.4%
TPFA +	14.7%	11%	8.7%	38%	6%
Combine +	13.5%	9.8%	10.2%	38%	5%
BFPs	2.9%	3%	0.3%	11.8%	1.3%

DISCUSSION

In the present study, the most common age group was of 21-30 years (38%), correlating with other studies by Manju Bala, et al 20-29yr (38%), Markos Negash et al 22-32yr (77%), where similar results were witnessed.^{7,8}

A male predominance was observed (63%), whereas the females were less in number (37%). It is in correlation with the study done by Sivakumar S. et al,⁹ Arti B. Ninama, et al¹⁰ where similar results were observed.

It is contrary to the study done by Murawala S et al¹¹ in which females were predominant.

In the current study, 6.4% were RPR reactive and 6% were TPFA positive cases, comparatively lower than the other studies done by M Bala et al.¹²

1.3% BFPs and 0.3% BFPs, were noted, this variation [probably due to study participants having acute/chronic infections or Autoimmune diseases.¹³

CONCLUSION

- Specific tests to diagnose syphilis like TPFA in combination with the non-specific test RPR will differentiate the true positives from BFPs.
- Rapid results and high sensitivity of RPR tests make it a good choice as a screening test in microbiology laboratories.
- RPR reactivity in high dilutions will indicate the activity of the disease and has got a prognostic value of the treatment
- Positive TPFA indicates past/present infection, and cannot be

used as an interpretative of successful or unsuccessful anti-treponemal therapy.

- The present study concludes that the TPHA test should be used for routine confirmation of a positive RPR test irrespective of its titer.

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