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Comparative Study Of Serological Test RPR &TPHA For Diagnosis Of Syphilis At A Tertiary Care Hospital, Guntur.

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Abstract
Background: *Syphilis, caused by Treponema pallidum, remains a significant public health issue, particularly for pregnant women, with the potential to cause severe maternal and fetal complications, including stillbirth, preterm birth, low birth weight, and congenital syphilis. Early diagnosis and treatment are essential to prevent these adverse outcomes.*
Objective: *This study aimed to assess the diagnostic accuracy of Rapid Plasma Reagin (RPR) and Treponema Pallidum Hemagglutination Assay (TPHA) tests in identifying syphilis in pregnant women and to evaluate the prevalence of biological false-positive results.*
Materials and Methods: *A prospective descriptive study was conducted from January 2025 to August 2025, involving 3,200 non-duplicate venous blood samples collected from antenatal care patients at Government General Hospital, Guntur. All samples were initially tested with the RPR test, and reactive cases were further tested using the TPHA. Data were analyzed with statistical significance set at a p-value of < 0.05.*
Results: *Out of 3,200 samples, 23 (0.71%) were RPR reactive. These samples underwent semi-quantitative RPR testing at dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. Higher dilutions (1:8 and above) showed a higher number of TPHA-positive cases, with 9 cases at dilutions >1:16. A total of 8 biological false-positive cases (0.25%) were identified in dilutions below 1:8. Among the 23 RPR reactive cases, the majority (60.87%) were in the 21-30 years age group, with 21.74% of false positives also occurring in this group. However, this age distribution was not statistically significant.*
Conclusion: *Syphilis diagnosis in pregnancy requires a combination of RPR and TPHA tests to minimize false positive results. Regular screening and early treatment are crucial to prevent syphilis-related complications. The study underscores the need for confirmatory testing in clinical practice to ensure accurate diagnosis and effective treatment, ultimately reducing maternal and neonatal morbidity and mortality associated with syphilis.*
Keywords: *Syphilis, RPR, TPHA, biological false positives, antenatal screening, pregnancy, Treponema pallidum.*

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I. Introduction

Syphilis is a sexually transmitted disease caused by the spirochete Treponema (T. pallidum) which affects 8 million adults globally acquired syphilis [WHO 2022]. Additionally, there were an estimated 700,000 cases of congenital syphilis. Despite the availability of relatively sensitive tests and affordable treatment, the disease remains a global health problem worldwide (Peeling and Hook, 2006), particularly for antenatal. When a pregnant woman is infected, syphilis can have severe consequences for both the mother and the foetus. Outcomes for the infant include stillbirth, neonatal death, prematurity, low birth weight, and congenital syphilis, which can cause long-term impairments. Infants may face hearing loss, vision problems, and cognitive issues if left untreated.[1] Syphilis also poses health risks to untreated pregnant women, increasing susceptibility to other STIs, including HIV, with a study by Arora et al., indicating a higher co-infection rate among pregnant women in India.[2] The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend routine syphilis screening during early prenatal care visits, with retesting in the third trimester and at delivery for high-risk women. [3,4]

Despite the availability of diagnostic tools, syphilis is often underdiagnosed during pregnancy, primarily due to the lack of visible symptoms in many infected individuals. This highlights the importance of consistent screening, particularly in regions with a high incidence of syphilis or within groups that are at greater risk.

While microscopy techniques (including dark field and immunofluorescence) and PCR can effectively identify spirochetes or their DNA in lesions, serology is still considered the most dependable approach for the laboratory diagnosis of syphilis, regardless of the infection stage. (Tsang et al., 2007). 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. Consequently, a comparative analysis of TPHA and RPR for the diagnosis of syphilis in antenatal care was conducted.

II. Materials And Methods

This study was conducted as a prospective study with IEC No:-GMC/IEC/036/2025 Dated:-20-01-2025 from January 2025 to August 2025.The confidentiality of all data was strictly maintained. All samples were initially tested using the Rapid Plasma Reagin (RPR) test. All RPR reactive samples were then further tested using the TPHA test.

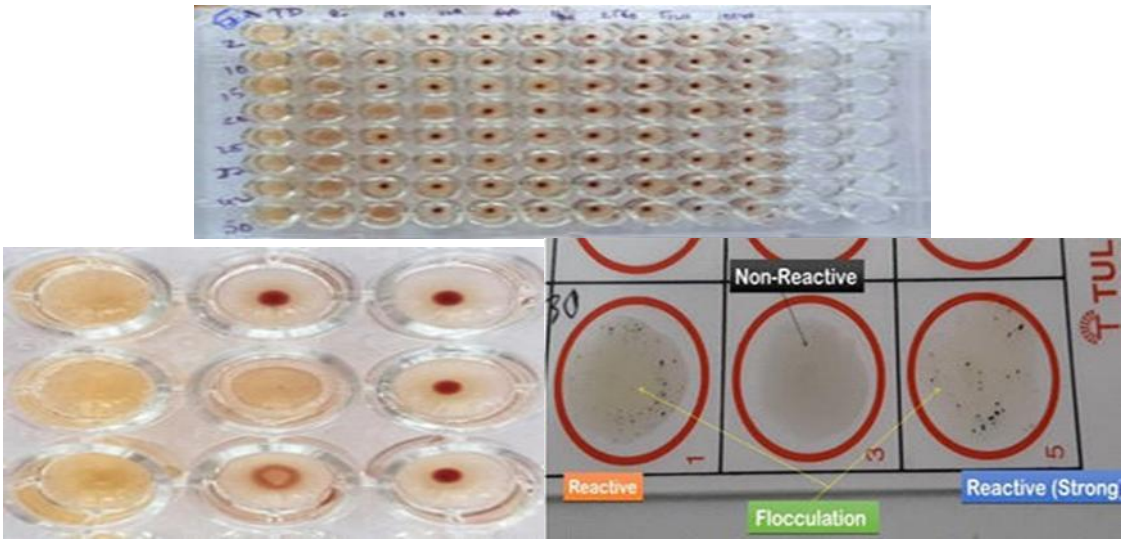
Inclusion Criteria: A total of 3200 non-duplicate venous blood samples were collected from Antenatal attending the outpatient department (OPD) at Government General Hospital, Guntur.
All samples were collected under aseptic precautions and transported to the microbiology lab.
A standard proforma was used to gather the medical and demographic data of the patients.

Exclusion Criteria: Samples with insufficient volume, hyperlipidemic samples. Statistical analysis was performed by SSPN and a p-value of less than 0.05 was considered statistically significant.

Procedure

For RPR testing, a venous blood sample of 5 ml was drawn into a plain vacutainer, allowed to clot for approximately 10 to 15 minutes, or centrifuged for 8 minutes at 2000 g.Before use, the antigen dispensing bottle should be gently shaken.

The RPR test involved mixing one drop of serum with one drop of RPR reagent on a white reaction card from the RPR test kit and placing it on a shaker for 8 minutes; results should be assessed in good lighting. A reactive sample is shown by the presence of visible black clumps against a white card background, while non reactive samples display a smooth, uniform light gray color. Results were classified as positive or negative based on the included positive and negative control sera for each test. The TPHA test was performed qualitatively, with an even layer of agglutinated cells at the bottom of the microtitration plate well indicating a positive result. In contrast, if the cells remain nonagglutinated, forming a compact button, it suggests a negative reaction. If agglutination occurs in both the control cell well and the test cell well, it indicates non specific agglutination in the sample, rendering the test invalid.



III. Results

From a total of 3200 patient samples, only 23 (0.71%) tested reactive to the RPR. Each reactive sample then underwent quantitative RPR testing with dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. Our dataset, detailed in Table 1, lists the RPR dilution levels, the number of reactive cases, and their outcomes concerning TPHA positivity and negativity. Higher dilutions of RPR, specifically those above 1:16, indicated a greater occurrence of TPHA-positive cases (n=9). At the 1:8 dilution level, 5 patients were TPHA positive, while 2

were TPHA negative. Moreover, eight cases of biological false positives were identified at dilutions lower than 1:8 using the semi-quantitative RPR test. No TPHA-positive outcomes were found in dilutions below this level. Biological false positives represented 0.25% of the total samples (8 out of 3200). Among the 23 reactive RPR cases, 14 (60.87%) belonged to the 21-30 year age bracket. Meanwhile, 5 (21.74%) of the false positive cases were found within the same age group (see Table 2). However, the disparity is not statistically significant, suggesting that false positive results can arise in any age group.

Table 1: RPR test results in antenatal

RPR reactive cases (%)	**Semi quantitative dilution**
1(4.35)	1:2
6(26.08)	1:4
7(30.44)	1:8
4(17.39)	1:16
4(17.39)	1:32
1(4.35)	1:64
Total (n= 23)	

Table 2: Age wise distribution of semi-quantitative RPR

RPR dilution	**< 20 yrs**	**21-30 yrs**	**31- 40 yrs**	**>40yrs**
1:2	0	1	0	0
1:4	1	4	1	0
1:8	1	4	2	0
1:16	1	2	1	0
1:32	1	2	1	0
1:64	0	1	0	0
(n= 23)	4	14	5	0

Table 3: Age wise distribution of semi-quantitative

RPR dilution	**< 20 yrs**	**21-30 yrs**	**31- 40 yrs**	**% TPHA Positive**
1:2	0	0	0	0
1:4	0	1	0	1(6.67)
1:8	1	2	2	5(33.34)
1:16	1	2	1	4(26.66)
1:32	1	2	1	4(26.66)
1:64	0	1	0	1(6.67)
				15

Table 4: Age wise distribution of semi-quantitative RPR test reactive and TPHA Positive in antenatal.
RPR test reactive and TPHA Negative in antenatal.

RPR dilution	**< 20 yrs**	**21-30 yrs**	**31- 40 yrs**	**% TPHA Negative**
1:2	0	0	1	1(12.50)
1:4	1	3	1	5(62.50)
1:8	0	1	1	2(25.00)
1:16	0	0	0	0
1:32	0	0	0	0
1:64	0	0	0	0
				8

Table 5: RPR and TPHA tests results in suspected syphilis cases.

Semi quantitative RPR dilution	RPR reactive (%)	TPHA positive, (%)	TPHA negative, (%)
1:2	1(4.35)	0	1(12.50)
1:4	6(26.08)	1(6.67)	5(62.50)
1:8	7(30.44)	5(33.34)	2(25.00)
1:16	4(17.39)	4(26.66)	0
1:32	4(17.39)	4(26.66)	0
1:64	1(4.35)	1(6.67)	0
	23	15	8

Additionally, 4 (17.39%) of the false positive cases were also in the same age group.

IV. Discussion

Syphilis during pregnancy can lead to severe complications such as spontaneous abortion, stillbirth, and health issues in infected infants. Effective treatment can prevent these outcomes, but identifying infected women is crucial. Screening in the first trimester with non-treponemal tests (RPR or VDRL) and confirming with treponemal tests (TPHA or FTA-ABS) is recommended. High-risk women should be retested in the third trimester. Penicillin is the preferred treatment, with dosage based on infection stage and HIV status. Desensitization is necessary for penicillin-allergic women. Despite treatment, 14% of pregnancies may still end in fetal death or the birth of an infected infant, and complications like the Jarisch-Herxheimer reaction can occur, leading to fetal distress. Pregnancy does not change the clinical progression of syphilis, but pregnancy-related cervical changes may facilitate the entry of spirochetes. The false positivity rate for syphilis screening tests is about 1%, with higher rates in specific groups such as the elderly, pregnant individuals, and those with autoimmune diseases or viral infections. A study found a 0.25% rate of biological false positives, emphasizing the importance of TPHA in diagnosing syphilis, as relying solely on non-treponemal tests would lead to even higher false positive rates. Specific treponemal serological tests identify antibodies against Treponema antigens but remain positive for life, limiting their utility in evaluating anti-treponemal therapy success. Although TPHA is not 100% sensitive and specific, its simplicity makes it preferable in less-equipped laboratories, especially in resource-limited settings. Semi-quantitative RPR test results at a dilution of 1:8 or higher can be considered equivalent to TPHA for syphilis diagnosis in these facilities.

V. Conclusion

A single non-treponemal antibody test, like RPR, is not conclusive for diagnosing syphilis due to its detection of reaginic antibodies, which may not indicate the disease's active stage. False positives can arise from various physiological factors and other infections, making specific treponemal tests necessary. Confirmatory tests, such as TPHA, are essential, and serology results must be interpreted alongside the patient's clinical presentation.

References

[1]. World Health Organization(WHO)."Syphilis." WHO, 2021.Www.Who.Int. Https://Www.Who.Int/News
[2]. Arora, R., Et Al. (2016)."Co-Infection Of Syphilis And HIV In Pregnant Women: A Study From India." Journal Of Medical Microbiology, 65(3), 150-155.
[3]. Centers For Disease Control And Prevention (CDC). "Syphilis And Pregnancy." CDC, 2023. Https://Www.Cdc.Gov/Std/Syphilis/Stdfact Syphilis- Pregnancy.Htm.
[4]. Newman L, Et Al. "Global Burden Of Syphilis In Pregnancy: A Systematic Review." The Lancet Infectious Diseases, Vol. 18, No. 2018, Pp. 770-779.
[5]. Wendel G. Gestational And Congenital Syphilis. Clinperinatol1988; 15:287–303.
[6]. Phaosavasdi S, Snidvongs W, Thasanapradit P, Et Al. Rapid Plasma Reagin Test (RPR) Compared To Venereal Diseases Research Laboratory Test (VDRL) For The Diagnosis Of Syphilis In Pregnancy. J Med Assoc Thai 1989; 72: 202–6.
[7]. Jaffe H, Larsen S, Jones O, Et Al. Hemagglutination Tests For Syphilis Antibody. Am J Clinpathol 1978; 70: 230–3.
[8]. Huber T, Storms S, Young P, Et Al. Reactivity Of Microhemagglutination, Fluorescent Treponemal Antibody Absorption, Venereal Disease Research Laboratory, And Rapid Plasma Reagin Tests In Primary Syphilis. J Clin Microbiol 1983; 17: 405–9.
[9]. Tankhiwale SS, Naikwade SR. Seroprevalence Of Syphilis And Biologically False Positive Cases In A Tertiary Care Center. Indian J Dermatol Venereol Leprol 2014; 80:340.
[10]. Hussain T, Kulshreshtla KK, Yadav VS, Sengupta V, Katoch VM. Seroprevalence Of Human Immunodeficiency Virus And Syphilis Infections Among Patients Attending The STD, Gyn. And Obs. ANC Clinics Of Northern India: Observations From Seven Rounds Of Sentinel Surveillance (1998 2004). Am J Infect Dis 2006;2: 28 35.