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Comparative Study of Serological Test RPR & TPHA for Diagnosis of Syphilis at Tertiary Care Hospital

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A B S T R A C T

Diagnostic and therapeutic approaches in syphilis show wide variation. The use of only one type of serological test is insufficient for diagnosis. To determine diagnostic performance of TPHA (*Treponema pallidum* Hemagglutination Assay) and RPR (Rapid plasma regain) test for syphilis. A study of syphilis was done over a period of April 2012 to March 2013 at Tertiary Care Hospital, Ahmedabad. A total of 1325 serum samples from clinically suspected cases of syphilis were sent to the microbiology laboratory. Screening test was carried out by RPR test and all positive tests were confirmed by TPHA. From total of 1325 cases, 410 sero positive cases in which285 patients were positive by both RPR and TPHA tests.51 cases (12.4%) which were positive by RPR and Negative by TPHA were later found that they all were false positive due to viral and malarial infection. TPHA is gold standard for the diagnosis of syphilis as compared to RPR/VDRL test. TPHA should be performed along with RPR test as no single serological test can act as the marker of acute infection.

Introduction

Sexually transmitted diseases are a major public health problem in India. Syphilis is one of the most fascinating diseases of humans. The disease has been of great historical importance not only for the practice of medicine but also because of its effect on many individuals who played important roles in the history of the western world. Syphilis is a sexually transmitted disease caused by the spirochete Treponema (*T. pallidum*) which affects 12 million people each year and results in significant morbidity and mortality (Sithamahalakshimi *et al.*, 2017).

Despite the availability of relatively sensitive tests and affordable treatment, the disease remains a global health problem (Peeling and Hook, 2006). Although the spirochetes or their DNA can be consistently detected in lesions by either microscopy (dark field, immunofluorescence) or PCR, the most reliable method for laboratory diagnosis of syphilis, regardless of the stage of infection, is still serology (Tsang *et al.*, 2007).

The non-treponemal tests such as RPR or VDRL test measure the host response to non-treponemal antigens such as cardiolipin and lecithin released from the damaged host cells, as well as lipoprotein-like material released from the treponema. These non treponemal tests are generally considered to be sensitive in early syphilis, but their disadvantages being false positive reaction. False-negative reactions due to the prozone phenomenon and lack of sensitivity in the late stage of infection. These tests are used for rapid screening of patients in field camps, blood donation camps and for assessing the effectiveness of therapy in syphilis patients.

The treponemal tests such as the *Treponema pallidum* Haemagglutination Assay (TPHA) and Micro haemagglutination assay for *Treponema pallidum* have high sensitivity for all the stages of disease other than very early primary syphilis. These tests detect human serum/plasma antibodies to *T. pallidum* by means of an indirect hemagglutination method (Monojit Paul and Subhrendu Sekhar Sen, 2016).

Biological false positive reaction define as positive results in non treponemal tests, with negative results in treponemal tests, in absence of syphilis and not caused by technical faults. (Essentials of Medical Microbiology) Confirmatory treponemal tests like TPHA are expensive and require more technical expertise compared to non-treponemal test. These tests are frequently not available in resource limited health care facilities and laboratories in developing nations like India. They are performed only in reference laboratories and results may not return for days. This scenario yields a biological false positive (BFP) results in patients without syphilis and may compromise clinical decision-making. Objective of these studies was to compare RPR test and TPHA in serological diagnosis of syphilis.

Materials and Methods

The retrospective study was carried out at Sheth V.S General Hospital, Ahmedabad, a tertiary care centre of Ahmedabad. Total 1325serum samples were collected for diagnosis of Syphilisat Integrated Counselling and Testing Centre (ICTC) and history was taken for all patients at STD and ANC clinic for clinical approach within the period of April 2012 to March 2013. Samples reactive for RPR has been further confirmed by TPHA test periodically.

The RPR test was performed qualitatively for all clinically suspected cases of syphilis and quantitatively only for titration of reactive samples. For RPR testing 5 ml venous blood sample was collected into a plain vacutainer, allowed to clot for about 10 to15 minutes or centrifuged for 8 minutes at 2000 g. A standard RPR test was carried out, by mixing one drop of serum with one drop of RPR reagent, on a shaker for 8 minutes, and results read in good light.

A Reactive sample is indicated by macroscopically visible black clumps against white background on card whereas non-reactive samples appear to have smooth uniform light gray colour. Results were recorded as positive and negative with respect to positive and negative control sera which were included in each test run.

The TPHA test was also performed qualitatively, wherein an even layer of agglutination cells in a round bottom of micro-titration plate well was interpreted as positive reaction, while non-agglutinated cells in case of absence of antibody form compact button which was interpreted as a negative reaction. Agglutination in the control cell well together with the test cell well indicates the presence of nonspecific agglutination in the sample, it is considered as the test was invalid.

Results and Discussion

Serum sample of 1325 suspected patients of syphilis were collected from ICTC from the person who attended the STD and ANC clinicat Sheth V. S Hospital, Ahmedabad. The study includes 410 patients with the positive diagnosis which was done by clinically and by laboratory methods of Rapid Plasma Reagin (RPR) test as screening test and confirmed by Treponemal Palladium haem Agglutination (TPHA) test.

Out of these 410 sero positive cases, gender distribution shows about 231(56.3%) were male which is preponderance than female of having 179 (43.6%) (Fig. 1). 158 (38.5%) cases had taken primary education, 115(28.04%) had taken secondary education and 52(13.5%) were having with higher education. about 85 (20.7%) cases were illiterate in this study population.

According to age wise distribution, maximum 162(39.5%) cases were identified in the age of 31 - 40 years followed by 123 (30%) in 21-30years, 77 (18.7%) in 41-50years, 28 (6.8%) in 51-65 years, 19 (4.6%) in 11-20 years, 1 (0.6%) in <10 years (Fig. 2).

Both RPR and TPHA tests were reactive in 285 patients.51 cases (12.4%) which were positive by RPR and Negative by TPHA were later found that they all were false positive due to viral and malarial infection. The TPHA test was performed for 74 RPR non-reactive samples, Out of these 51 sera samples were positive by TPHA. 17 were late syphilis cases and 6 were fully treated syphilis cases.

Serodiagnosis of syphilis occupies an important place in any diagnostic laboratory. The commonly used screening tests are the non treponemal tests, employing cardiolipin antigen. These tests are non-specific. Inspite of this, they are used widely and are preferred by clinicians and diagnosticians because they are affected by anti-treponemal therapy. As a result they are useful for following the progression of the disease and response to therapy.

As mentioned in the literature, specific treponemal serological tests detect treponemal antibodies against the antigens of the organism themselves. Once positive, their usefulness is limited because these tests tend to yield positive results throughout the patient's life. TPHA test cannot distinguish between syphilis and other pathogenic treponemal infections like Yaws.

The positive result with TPHA can be indicative of an ongoing or a past infection. Thus TPHA cannot be used as interpretative of successful or unsuccessful anti-treponemal therapy. Although TPHA is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders (Lesinski *et al.*, 1977). TPHA carries more advantages when compared to the other treponemal tests, and is more laboratory-friendly (Table 1).

Table.1 Comparison of results by RPR and TPHA tests

Results	RPR+TPHA(n=410)	RPR(n=410)	TPHA(n=410)
Reactive	285(70%)	336(82%)	364(88%)
Non-reactive	125(30%)	74(18%)	46(12%)

Fig.1 Gender distribution of sero positive

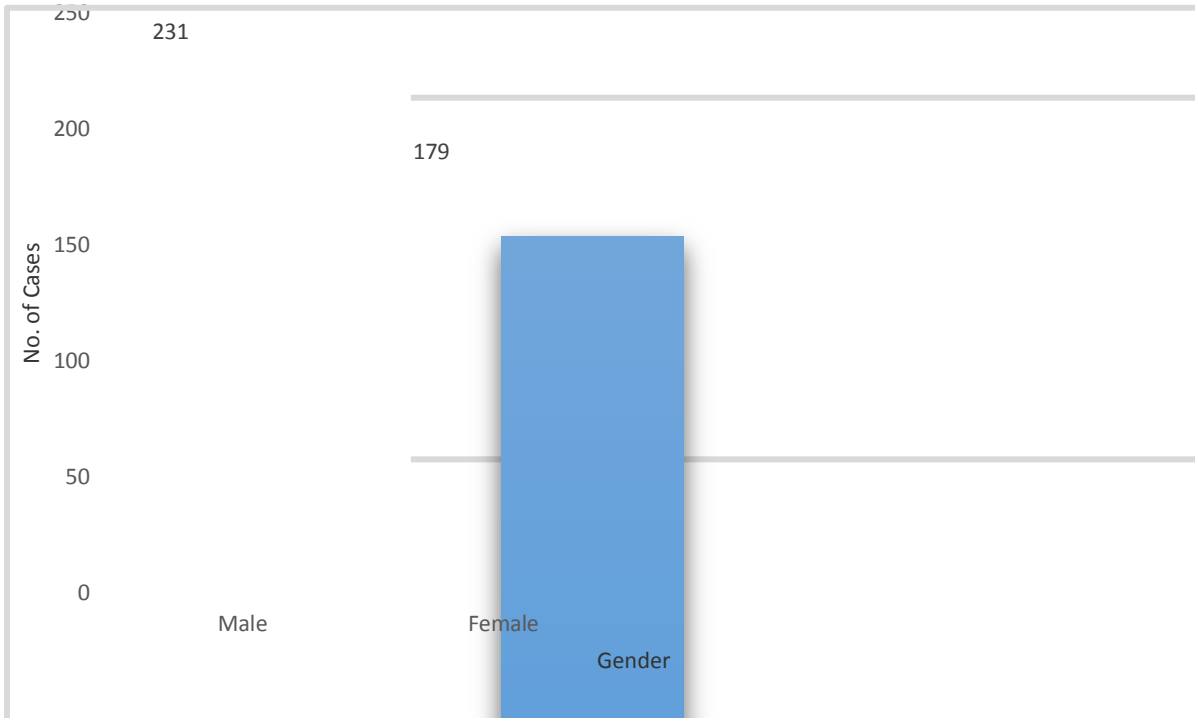
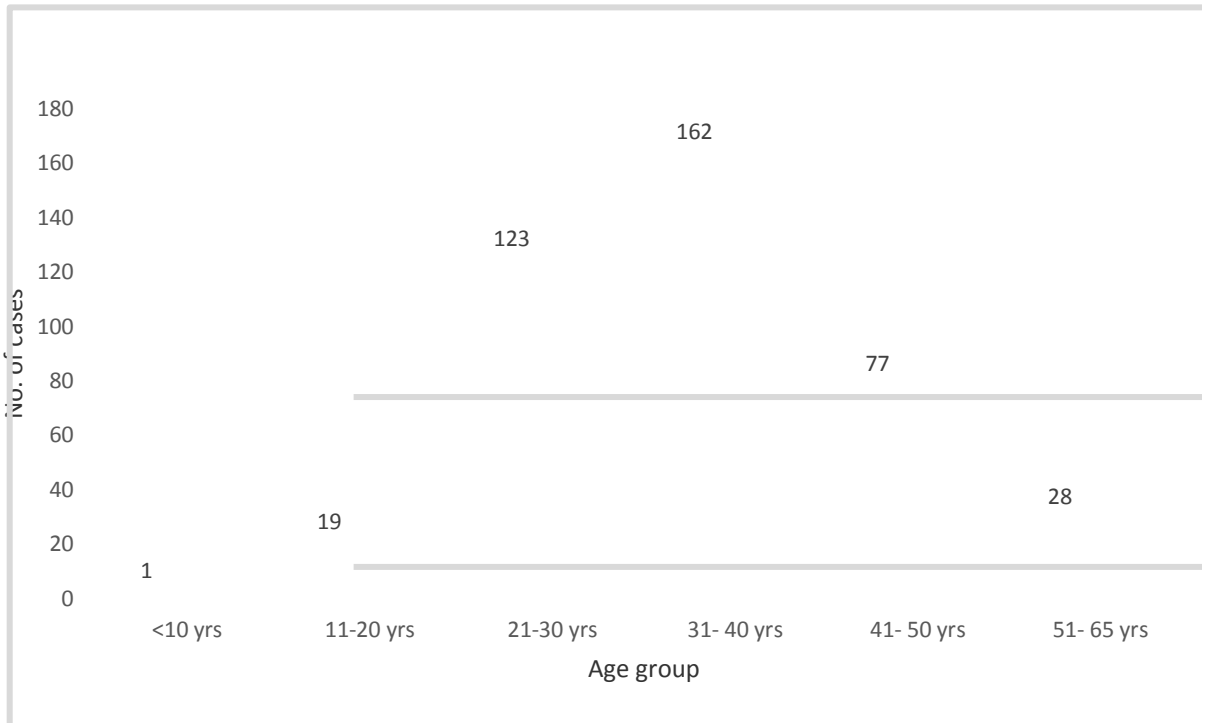


Fig.2 Distribution of cases in age group



Diagnostic and therapeutic approaches in syphilis show wide variation (Tasbakan *et al.*, 2008). The use of only one type of serologic test is insufficient for diagnosis because each type of test has some limitations (Centers for Disease Control and Prevention, 2010). The sensitivity of non-treponemal tests depends on the stage of syphilis (Sambri and Lautenschlager, 2006). The traditional approach to syphilis sero-diagnosis utilizes a two-step approach: first screening with a non-treponemal test such as the rapid plasma reagin (RPR) test and then performing confirmatory testing on those specimens reactive in the screening test using a treponemal test such as the *T. pallidum* Haemagglutination assay (TPHA). All non-treponemal serologic tests measure antibodies to cardiolipin. Generally, these tests take the form of flocculation reactions.

In this study, 56.3% Male were more affected than female and According to age wise distribution, maximum 162(39.5%) number of seropositive cases were identified in the age group of 31 years to 40 years which correlate with study of (Monojit Paul and Subhrendu Sekhar Sen).

81.6% of RPR reactive results were confirmed by TPHA test as compared to only 73.2% of RPR reactive results were confirmed by TPHA test in study of SP Dumre (Dumre *et al.*, 2011). Single test of non-treponemal antibody like RPR and VDRL should not considered as confirmative due to following reasons: It detects only the reaginic antibodies which do not conclusively prove the active stage of the disease, the occurrence of biological false positivity due to physiological conditions and certain acute and chronic infections, biological false negative results in late and latent syphilis. So, confirmation of RPR results should be done by TPHA or other specific treponemal tests in all patients to ensure that appropriate syphilis diagnosis has been made as mentioned in the national STI management guidelines (National Centre for AIDS and STD Control, 2006).

In this study, 88% of the TPHA positive cases were positive by RPR test as compared to only 80.4% and 91.08% in study of (Dumre *et al.*, 2011), (Murawala *et al.*, 2015) respectively. TPHA as a treponemal antibody test does not satisfy as confirmatory test as it lacks the sensitivity in sera from patients with primary syphilis. 20% positive TPHA test were negative in RPR which same as in study of (Dumre *et al.*, 2011). TPHA demonstrated relatively less false positive results compared to RPR in our study. Such TPHA positive cases with RPR negative result were found as treated syphilis cases and some as late syphilis cases which indicates more specificity of TPHA test.

Based on our results we think that specific Treponemal tests could contribute to reducing errors that depend on specificity of the method used. Considering the methodology, rapid results and high sensitivity of RPR tests makes it a good choice as screening test in microbiology laboratories. The limits of screening tests for the diagnosis of syphilis should not be forgotten, i.e. confirmatory tests like TPHA must be done. Serology has the prime importance in the laboratory diagnosis of syphilis, but must be viewed in the context of clinical presentation.

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