"Syphilis Seropositivity in patients attending a tertiary care hospital, Mysuru, South India"

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Abstract:

Introduction: Syphilis a multi-system disease caused by a spirochete Treponema pallidum subspecies pallidum. In recent years, decline in its prevalence has been observed in the world however in the developing countries like India, syphilis still continues to be a major public health problem. Difficulties in the diagnosis of syphilis continue to challenge clinicians. The incapability to readily culture T. pallidum has forced laboratorians to focus on alternative methods for diagnosing syphilis. Serological tests are the main stay for the rapid diagnosis of this condition.

Aims and Objectives: The study was undertaken to know seropositivity of syphilis by RPR test in a tertiary care hospital and to compare the diagnostic value of RPR and Treponema pallidum hemagglutination test (TPHA) for the laboratory diagnosis of Syphilis.

Results: A total of 1930 patient's blood specimens were analysed from patients using RPR (Rapid plasma reagin) test. The RPR positive samples were tested by TPHA. The seropositivity rate of syphilis by RPR was found to be 18 (0.93%). The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of RPR test were found to be 100 %, 66.67%, 50%, 100% and 75% respectively in comparison to TPHA. Conclusion: Though RPR is routinely used as a screening test, the gold standard specific test like TPHA must be done to confirm Syphilis and to initiate specific treatment early and to prevent the complications.

Key words: Syphilis, Treponema pallidum, RPR, TPHA

INTRODUCTION:

Syphilis, a sexually transmitted infection is known to be caused by Spirochete group of bacteria, Treponema pallidum subspecies pallidum. The spectrum of this disease ranges from being asymptomatic to disseminating into severe complications which may involve cardiovascular and central nervous system1. Estimation of the disease burden is challenged globally due to sparse availability of data. This might be because of social stigma, lack of diagnostic facilities and failure in detection of asymptomatic individuals2. Several studies have documented an increase in the incidence of the disease from last twenty years3-6 and there is drastic raise of the cases among men having sex with men (MSM)7. Unsafe sexual practices, homosexuality and co-infection with HIV have attributed to increase in the rate of infection. Proper medical history and presence of clinical manifestations, signs and symptoms along withdirect detection of the causative agent from the clinical specimens or a positive test for treponemal and non-treponemal tests is required for accurate diagnosis of Syphilis[8,9].

Three common approaches for serological diagnosis of Syphilis were recommended by CDC. Firstly, for screening a non-treponemal assays like Rapid plasma reagin (RPR) test or a Veneral Disease Research laboratory (VDRL) test can be employed to identify the population with possible infection. These screening assays has to be followed by Treponemal confirmatory assays such as Treponemal hemagglutination assay (TPHA) or by Fluorescent treponemal antibody absorption test (FTAABS). In second, the above algorithm was updated in a reverse sequence which is used to assess the disease, treatment status and for confirmation. These tests are based on the availability of automated assays like Enzyme immune assays and Chemiluminescence immunoassays.. If the specimens are found to be positive by above tests, a quantitative assay or different treponemal assay can be used. Thirdly, there is the European Centre for Disease Prevention and Control (ECDC) algorithm, which starts with a primary treponemal screening test followed by a second, different confirmative treponemal assay[10-12.] There is a very limited information regarding the seropositivity of Syphilis in Mysuru city , a district in South Karnataka. Hence a

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laboratory based prospective study was conducted to determine the rate Syphilis seropositivity among the suspected cases attending a tertiary health care centre located in Mysuru.

MATERIALS AND METHODS:

This is a laboratory based prospective study conducted in the Department of Microbiology, JSS Hospital for a period of 1 year after obtaining the approval from the Intuitional ethics committee. The serum samples received in the department for diagnosis of Syphilis or for screening through RPR were also subjected TPHA assay as per the standard protocol. 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 were read in good light. A reactive sample was indicated macroscopically by visible black clumps against white background on card whereas non-reactive samples appear to have smooth uniform light grey colour. Results were recorded as positive and negative in comparison to positive and negative control sera which were included in each test run. The TPHA test was also performed on 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 was considered as invalid. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of the tests were calculated using SPSS software.

RESULTS:

Blood samples from 1930 patients with suspected syphilis that were received in the Department of Microbiology, JSS Hospital, Mysuru were included in the study. Serum obtained from the blood samples were subjected to RPR test as screening assay and TPHA as confirmatory assay respectively. Of 1930 specimens processed 18 (0.93%) were positive by RPR test. 9 (50%) of the samples that were positive by RPR were also positive by TPHA. 7 (77.8%) of the samples were from males and 2 (22.2%) were from females. Mean age of the patients who tested positive by at least one of the two tests, RPR and TPHA was 29 years. The sensitivity, specificity and predictive values of RPR in this study were evaluated by using TPHA as a gold standard for the diagnosis of syphilis. Thus, the sensitivity and specificity of RPR for syphilis detection were 100 and 66.67 %, respectively. The positive predictive value (PPV) and negative predictive value (NPV) were 50 and 100%, respectively. The Diagnostic accuracy of RPR test was 75%. Among TPHA positive (9) cases, most of the patients were from dermatology outpatient departments with 5 (55.56 %), followed by Urology and General medicine OPD each having 2 (22.22 %) respectively. The diagnosis among the patients who visited dermatology OPD includes Generalized lymphadenopathy, Chancre with secondary infection, Skin lesions, Hyperpigmentation lesion, Left hemiplegia secondary to right MCA territory infarct with skin lesions. The patient from General medicine dept were diagnosed with Cerebrovascular accident (CVA) and Dizziness with myalgia. The patients attending urology were diagnosed with Left chronic epidydimoorchitis with secondary hydrocele and Left hydrocele epididymitis.

DISCUSSION:

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 monitoring the disease progression of the disease and response to therapy. Of 19 positive cases in our study, gender distribution showed males (77.7%) predominating and among 9 cases that were positive by RPR and TPHA females predominated with 53.85%. This is in concordance with a study conducted by Arti B. Ninama et.al13. 9 (50%) samples in our study were positive by both RPR and TPHA.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 disorders14. TPHA carries more advantages when compared to the other treponemal tests, and is more laboratory-friendly. Diagnostic and therapeutic approaches in syphilis show wide variation. The use of only one type of serologic test is insufficient for diagnosis because each type of test has some limitations (CDC, 2010). Sensitivity and specificity of RPR test in our study was 100 and 66.7% respectively. The sensitivity of non-treponemal tests depends on the stage of syphilis15. 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 and are flocculation reactions. Although clinical diagnosis of syphilis is easier in primary syphilis, the diagnostic tests are of great help in diagnosis and management of secondary or tertiary syphilis where characteristic clinical features are not present.

CONCLUSION:

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.

ETHICAL CLEARENCE: This current study is approved by the Institutional ethics committee of JSS Medical college and Hospital, JSSAHER, Mysore.

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CONFLICT OF INTEREST: Authors declare no conflict of interest.

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