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USE OF TPHA AND VDRL IN THE DIAGNOSIS OF SYPHILIS

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Abstract

INTRODUCTION: Venereal Disease Research Laboratory (VDRL) test is performed by the physicians to screen patients for syphilis and is still the most commonly used test all over the world for screening. Three basic methods has been described in screening for syphilis. These include direct observation of the T. pallidum by dark field microscopy, and nontreponemal and treponemal serologic antibody studies. Nonspecific (non tremonemal) tests like Venereal Disease Research Laboratory (VDRL) test use lipoidal antigens containing cardiolipin, lecithin, and cholesterol, that flocculate with IgM and IgG. Positive tests occur from 21 days of exposure till about up to 6 weeks after infection. Serologic tests in syphilis only provide indirect evidence of syphilis and may be reactive in the absence of clinical or epidemiologic evidence of syphilis. The reactivity in these cases is usually in low dilutions (<1:8), however, in exceptional cases false reactivity is shown in very high titers up to 1:256. False-positive reactions can also also be seen with treponemal tests.

MATERIAL AND METHODS: A total of 10253 patients were tested for syphilis by VDRL as a screnning test during study period. Both the the qualitative and quantitative VDRL tests were done as per the manufacturer's instructions. TPHA was performed on all the sera demonstrating reactivity with VDRL test. VDRL test was based on the principle that after syphilis infection, host develops nontreponemalantilipoidal antibodies in response to the release of lipoidal material from the damaged host cells. Also host produces antibodies against T. pallidum. In TPHA Agglutination of cells shows a positive reaction. In the absence of antibody i.e. in negative cases cells settled down to form a compact button in the well which constituted a negative reaction.

RESULTS: In this study a total of 10253 patients were screened for syphilis by VDRL of which 98 were reactive. In a sample of 98 VDRL reactive patients, 33(34%) were males and 65(66%) females. The age of patients who tested VDRL positive in our study ranged from youngest being 21 years to eldest being 66 years. Majority of patients i.e. 88.8% belonged to 20-50 years of age group, with majority of patients 37.75% belonging to age group 30 – 40.

CONCLUSION: VDRL test is the best screening test for the diagnosis of syphilis but whenever the patient is serologically reactive it should be confirmed by the more specific TPHA test.

Introduction

Venereal Disease Research Laboratory (VDRL) test is performed by the physicians to screen patients for syphilis and is still the most commonly used test all over the world for screening. Three basic methods has been described in screening for syphilis. These include direct observation of the T. pallidum by dark field microscopy, and nontreponemal and treponemal serologic antibody studies. Sensitive nontreponemal tests such as the VDRL and the rapid plasma reagin (RPR) are used for initial screening, whereas specific treponemal tests such as the fluorescent treponemal antibody absorption (FTA-ABS) are used as confirmatory tests[i]. Syphilis infection is a contagious systemic disease having sequential clinical stages and having years of latency. The infection which is caused by the bacteria *Treponemapallidum*, is endemic in several parts of the world and continues to remain a public health concern[ii].

Nonspecific (non tremonemal) tests like Venereal Disease Research Laboratory (VDRL) test use lipoidal antigens containing cardiolipin, lecithin, and cholesterol that flocculate with IgM and IgG. Positive

tests occurs from 21 days of exposure till about up to 6 weeks after infection[iii]. VDRL is inexpensive, simple, and suitable for mass screening and the baseline titer can be used to follow-up the treatment response. But the conformational tests are required as the sensitivities and specificities of nontreponemal tests vary with the stages of infection and also to rule out biological false positive reactions[iv].

Serologic tests in syphilis only provide indirect evidence of syphilis and may be reactive in the absence of clinical or epidemiologic evidence of syphilis. The reactivity in these cases is usually in low dilutions (<1:8), however, in exceptional cases false reactivity is shown in very high titers up to 1:256. False-positive reactions can also alsobe seen with treponemal tests, but this is less common than with nontreponemal tests hence, careful clinical interpretation of test results and other evidence is necessary for proper diagnosis.[v].Also it is recommended that serology should be repeated at 10 weeks, till most cases become VDRL non-reactive[vi].

MATERIAL AND METHODS

Present study was carried out in the department of Microbiology in collaboration with department of Medicine. This study was done in the Ananta Institute of Medical Science and Research Centre Rajsamand . A total of 10253 patients were tested for syphilis by VDRL as a screnning test during study period. Both the the qualitative and quantitative VDRL tests were done as per the manufacturer's instructions. TPHA was performed on all the sera demonstrating reactivity with VDRL test.

VDRL test was based on the principle that after syphilis infection, host develops nontreponemalantilipoidal antibodies in response to the release of lipoidal material from the damaged host cells. Also host produces antibodies against *T. pallidum*. These nontreponemal antibodies are eferred to as reaginsantibody which react with cardiolipin to give a flocculation reaction which can be observed under light microscope

TPHA test was based on *T. pallidum* sensitized formalized tanned fowl erythrocytes; unsensitized formalized tanned fowl erythrocytes and control sera. On mixing the diluted positive sera with sensitized erythrocytes, antibody to the sensitizing antigen led to agglutination of cells. Agglutination of cells shows a positive reaction. In the absence of antibody i.e. in negative cases cells settled down to form a compact button in the well which constituted a negative reaction[vii].

We categorised cases in to VDRL Positive, TPHA positive and Biological fase positive which were positive by VDRL and negative by TPHA test.

RESULTS

In this study a total of 10253 patients were screened for syphilis by VDRL of which 98 were reactive

Table 1: Demographic characteristics sex distribution

SEX	No. of patients
Male	33
Female	65

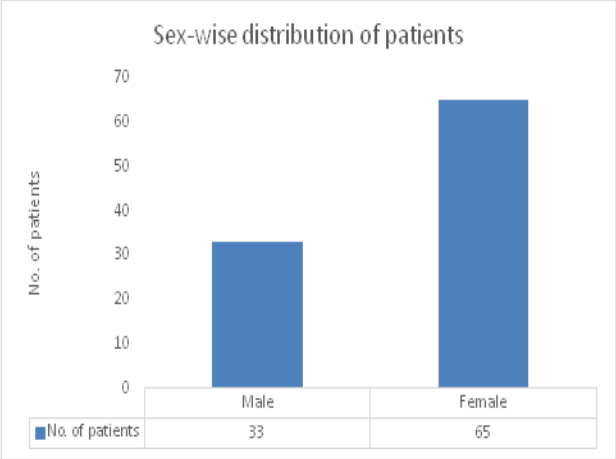

Figure 1: Male female distribution

In a sample of 98 VDRL reactive patients, 33(34%) were males and 65(66%) females.

Table 2: Age & sex wise distribution of VDRL reactive cases

Age	Male	Female
20 - 30	6	15
30 - 40	13	24
40 - 50	11	18
50 - 60	2	7
60 - 70	1	1
Total	33	65

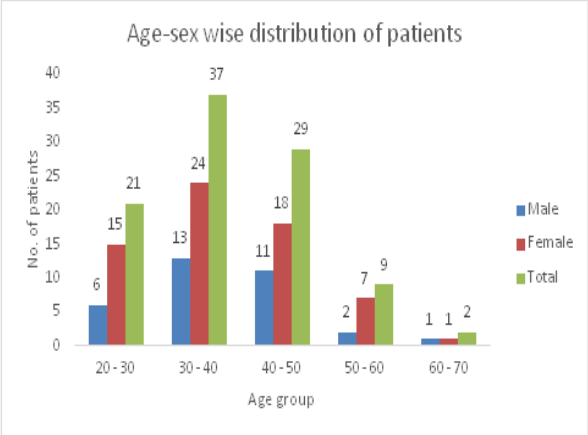

Figure 2: Age and sex distribution of the patient.

The age of patients who tested VDRL positive in our study ranged from youngest being 21 years to eldest being 66 years. Majority of patients i.e. 88.8% belonged to 20-50 years of age group, with majority of patients 37.75% belonging to age group 30 – 40. All male patients were referred from STD clinics whereas majority of female cases were referred from ANC clinic.

Table 3: VDRL and TPHA reactive cases

Test	VDRL
TPHA	Reactive
Positive	90
Negative	8
Total	98

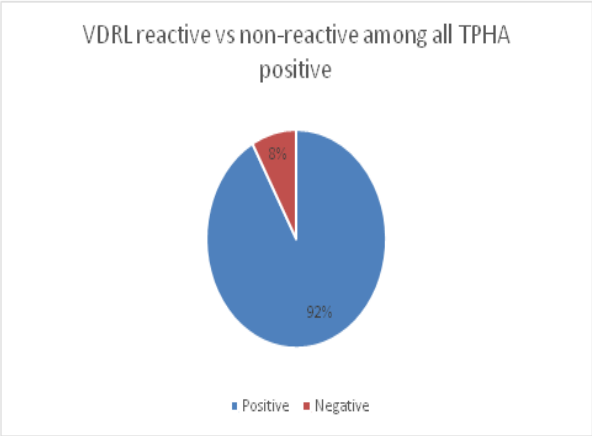

Figure 3: VDRL and TPHA reactive cases.

Out of 10253 serum samples tested during the study, 98(0.95%) samples were tested VDRL reactive. TPHA was performed on all VDRL reactive samples and was found to be positive in 90(97.8%) cases.

DISCUSSION AND CONCLUSION

There is a sensitivity of 70–90% in nontreponemal screening tests but it has to be confirmed by a more specific treponemal test. There is 100% positivity of serological tests at secondary stage and sensitivity for all tests including VDRL test which is approximately 100%; however, in 1–2% of patients' false-negative nontreponemal tests can occur due to prozone phenomenon. A presumptive diagnosis in patients is done based on the presence of typical rash and reactive non-treponemal tests in a titer ≥1:8 in a patient with no previous history of syphilis. If history of syphilis is present, then there should be a fourfold rise in titer. When a titer of nontreponemal test <1:8, the test should be repeated and confirmatory treponemal test should also be performed. Reactivity of the nontreponemal tests decreases in the period of latency, and in about 30% of the patients with late latent or late syphilis VDRL/RPR test are generally negative. Therefore in all patients with late latent syphilis, lumbar puncture is recommended to exclude neurosyphilis[viii]. Also it is found that the VDRL titre may not come down in patients with late syphilis and titre remains reactive at a low level (<1:8) for many years even after adequate treatment. Still there is no satisfactory monitoring test available for nontreponemal test-negative late disease[ix].

In the present study we screened 10253 cases for syphilis of which VDRL reactive were 98 (0.95%). In a study by Kashyap B et al [7]VDRL reactivity was found to be 0.77% among 22,351 patients.

Of the 98 VDRL reactive cases. 90 were positive by confirmatory TPHA test. So 8(8%) cases were reported as biological false positive (BFP) cases. The VDRL titres in BFP reactions ranged from 1:2 to 1:8. In other studies BFP reactions were low. In a study in Vienna General Hospital, the BFP reactivity was found to be 0.24% of all patients[x]. In a study of Saudi Arabia from obtained in pregnant women and blood donors, BFP were detected in 0.5% of the total sera examined, with 0.4 and 0.8%, respectively[xi].in our study high rate of BFP may be due to more number of pregnant ladies in the study group as pregnancy itself gives BFP reactions. In a study by Dheepa D et al [xii] reported a biological false positive (BFP) of 1.1% in their study.

Antenatal screening for syphilis is an effective method to reduce the consequences of congenital syphilisspecially on neonate as syplillis is congenitally transferred to the baby. Therefore, early serological screening for syphilis of antenatal case (ANC) is of paramount importance for commencing an early adequate treatment. In our study out of 89 VDRL reactive 60(61%) cases positive by VDRL were antenatal cases. Out of 38 non ANC cases 33 (86.8%) were male and 5(13.2%) were female.

Thus it is concluded that VDRL test is the best screening test for the diagnosis of syphilis but whenever the patient is serologically reactive it should be confirmed by the more specific TPHA test.

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