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Original Research Article

Assessment of Serological Tests for Syphilis in Patients Attending STD OP in A Tertiary Care Hospital

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Abstract: Syphilis is a chronic sexually transmitted infection diagnosed most commonly by serological methods. The serological methods of diagnosis of syphilis include non-treponemal and treponemal tests. The aim of this study was to assess the specific and non specific tests for syphilis and to know the frequency of biological false positive reactions in patients attending STD OP and the individuals attending the master health checkup. The seroprevalence of syphilis based on VDRL test was 2.54%. The prevalence of syphilis was more in STD clinic attendees 3.69%. Biological false positivity was 0.3% in patients attending STD OP and 0.44% in general population in our study. The Biological false positive reactions were more in the samples with < 1:8 dilutions. From our study, we suggest that VDRL test can be used as a screening test for the diagnosis of syphilis in patients attending STD OP. All the reactive VDRL samples should be confirmed by the treponemal TPHA test to exclude the biological false positive reactions.
Keywords: Syphilis, VDRL test, TPHA test, Biological false positive reactions.

INTRODUCTION

Syphilis is a chronic sexually transmitted infection caused by a spirochaete bacterium *Treponema pallidum*. It is a systemic infection capable of involving every structure of the body in its course, and distinguished by florid manifestations on one hand and years of completely asymptomatic latency on the other [1]. It can simulate many diseases and present to a wide range of medical specialities [2]. Infectious or early syphilis is important particularly when related to adverse pregnancy outcomes and the facilitation of HIV transmission by the lesions of primary and secondary syphilis [3].

Since *Treponema pallidum* is a non-cultivable bacteria, serology plays an important role in the diagnosis of syphilis. The serological diagnosis of syphilis is based on the antibody detection by non-treponemal and treponemal tests. Over a long period of time, the non-treponemal test, VDRL is being performed for routine screening for syphilis. Though the non-treponemal screening tests show 80% accuracy in the primary syphilis, they are not truly specific for syphilis. The VDRL test is rapid, simple and inexpensive when compared to the specific test like TPHA. It can be used for screening, to monitor the course of the disease and to detect reinfection.

Non-treponemal tests like VDRL and RPR lack sensitivity in early and late stage infection and screening with a non-treponemal test alone may also yield false positive reactions in various acute and chronic conditions in the absence of syphilis known as biological false-positive (BFP) reactions [4]. Biological false positivity will psychologically affect the patients and will lead to unnecessary treatment without any infection. All the reactive samples in VDRL test should therefore be confirmed with specific tests like TPHA. In clinically suspected syphilis patients, VDRL may be non reactive. They may be treated or late/latent case of syphilis. In those patients, the samples should be tested with TPHA test to rule out syphilis.

AIM OF THE STUDY

This study was done to assess the non-treponemal VDRL test and the treponemal TPHA test

for the diagnosis of syphilis and the frequency of biological false positive reactions in patients attending STD OP in a tertiary care hospital.

MATERIALS AND METHODS

This is a retrospective study done over a period of 6 months from May to October 2016 in a tertiary care hospital in Chennai. The samples were collected from patients attending STD OP and those who came for general screening in master health checkup scheme.

All the samples were subjected to VDRL test. The serum sample was inactivated at 56°C for half an hour. 50µl of sample, positive and negative controls were mixed with one drop of VDRL antigen in separate circles of VDRL slide. The slide is rotated in VDRL rotator at 180 rpm for 4 minutes. Then the slides were observed for flocculation under microscope. The VDRL antigen used was obtained from The Serologist, Government of India, and Kolkata. The reactive samples were subjected to qualitative VDRL test with two fold dilutions of the serum with saline.

All the sera reactive in VDRL test were confirmed for antibodies to *Treponema pallidum* by TPHA test. 25 µl of diluted serum sample was mixed with 75µl of control cells and test cells from the TPHA kit in separate wells of the TPHA plate and mixed well. Positive and negative controls were also included with the test serum. The plate was then incubated for 45-60 minutes at room temperature. The wells were then observed for agglutination. The kit used for TPHA kit was Omega diagnostic kit from Scotland, UK. The assessment was done by dividing the VDRL reactive samples into two divisions, one with < 1:8 dilution and the other with ≥ 1:8 dilution.

RESULTS

4330 samples were tested from the STD clinic attendees. Among the 4330 samples, 160 samples (3.69%) were reactive in VDRL test, 111 (2.6%) were male and 49 (1.1%) were female. VDRL reactivity was more in the age group of 21-45 years with 98 samples (2.26%) reactive in this age group. 53 samples (1.2%) were reactive in >45 years age group and 9 samples (0.2%) were in < 20 years of age. Among the 160 reactive samples, 136 samples (3.14%) were having titre < 1:8 whereas 24 samples (0.55%) were with titre ≥ 1:8. TPHA positivity was seen in 124 samples (2.86%) out of the 136 samples with < 1:8 titre and 12 samples (0.28%) were TPHA negative. From the 24 samples with ≥ 1:8 titre, 23 samples (0.5%) were TPHA positive and 1 sample (0.02%) was TPHA negative.

2896 samples were tested from the persons who came for general screening in master health checkup. 24 samples (0.82%) were reactive for VDRL with 22 samples (0.76%) in <1:8 titre and 2 samples (0.07%) in ≥ 1:8 titre. In these 24 reactive samples, 13 (0.44%) were in the age group of 21-45 years. 9 samples (0.31%) were TPHA positive and 13 samples (0.45%) were TPHA negative among the 22 samples with < 1:8 titre. The 2 samples with ≥ 1:8 titre were TPHA positive.

Overall the VDRL reactivity was 2.54% considering both the patients attending STD OP and general population. The VDRL reactivity was more (3.69%) in patients attending STD OP when compared to the cases from general screening where the reactivity was found to be 0.82%. The rate of Biological false positivity among the STD clinic attendees was 0.3% when compared to the general group with the rate being 0.45%. Among the 13 biological false positive samples in STD patients, 0.18% was in female and 0.11% was in male. Among the biological false positivity in general group, 0.34% was females and 0.1% were males.

Table: 1

Group tested	Samples tested	VDRL reactive – Male				VDRL reactive – Female			
		< 1:8		≥ 1:8		< 1:8		≥ 1: 8	
		TPHA Pos	TPHA Neg	TPHA Pos	TPHA Neg	TPHA Pos	TPHA Neg	TPHA Pos	TPHA Neg
STD clinic	4330	88	4	18	1	36	8	5	0
MHC scheme	2896	4	3	2	0	5	10	0	0

Table: 2

Group tested	Total Samples tested	VDRL Reactive	TPHA Positive	Biological false positive
STD clinic	4330	160	147	13
MHC scheme	2896	24	11	13

DISCUSSION

In this study, the seroprevalence of syphilis based on VDRL test was 2.54% which is similar to the study by Hossain *et al.;* in Saudi Arabia where it was 2.7% [5]. In another study in Jamaica, the seroprevalence of syphilis was 2.2% [6]. The prevalence of syphilis was more in STD clinic attendees 3.69% than the other group where it was 0.82%. This observation is similar to another study by Bala *et al.;* in Delhi where the prevalence of syphilis in STD patients was 3.5% [7]. The prevalence in STD clinic attendees is more due to their high risk behavior and this will increase the transmission of HIV also. Seroprevalence was highest in male attendees (1.66%), which is comparable to results seen in a Maharashtra study where the prevalence in men was 1.42% [8]. VDRL reactivity was highest in the age group of 21-45 years in both STD clinic attendees and general group which is the sexually active reproductive age group. But nowadays the prevalence of syphilis is increasing in the age group of < 20 years also.

Biological false positivity was 0.3% in STD clinic attendees and 0.44% in general population in our study. This is comparable with a study in Vienna where the BFP rate was 0.24% [9]. In a Jamaican study, the BFP reactions were detected in 0.59% of general population [6] and in a study in Saudi Arabia, the prevalence was 0.5% [5]. These findings are similar to another study by Moore *et al.;* [10]. In our study the Biological false positivity was more among females which was 0.2% than males which was 0.15%. This is similar to other studies in Vienna and Jamaica [6, 9]. But this is in contrast to a study by Bala et al, in which the BFP rate was more in males [7]. The increased rate in females may be due to the fact that chronic illness like systemic lupus erythematosus and other autoimmune diseases are more common in females. Pregnancy is also an important reason for the Biological false positivity in women.

In our study the Biological false positive reactions were more in the samples with < 1:8 dilutions. This is similar to the study conducted by Bala *et al.;* [7]. So the diagnosis of syphilis in patients with titre of < 1:8 should be done only after confirmation with TPHA. In exceptional cases, false positive reactions were seen in higher titre also [11] and so quantitative titre should not be used to differentiate biological false positive reaction and syphilis. Clinically suspicious patients with non-reactive VDRL should be tested by TPHA since they may have been fully treated for syphilis or may be in the latent or late stage of syphilis. They are likely to develop complications like cardio syphilis and neurosyphilis and the scar of syphilitic infection is shown by TPHA positivity [12]. From our study, we suggest that VDRL test can be used as a screening test for the diagnosis of syphilis in patients attending STD OP. All the reactive VDRL samples should be confirmed by the treponemal TPHA test to exclude the biological false positive reactions.

CONCLUSION

Non-treponemal tests like VDRL or RPR are commonly used in many places for the diagnosis of syphilis since they are rapid, simple and inexpensive. In many of the hospital settings, non-treponemal test is the only test done for the diagnosis of syphilis. Biological false positive reactions can occur in this type of screening tests which may lead to unnecessary treatment & can cause psychological trauma to the individuals without infection. BFP reactions are more common in < 1:8 dilution of VDRL than with higher titres. TPHA test which is a specific treponemal test should therefore be done to confirm the reactive VDRL samples to exclude the biological false positive reactions. TPHA test should be done for the non reactive samples of clinically suspicious patients also to detect the treated and latent/late cases of syphilis. In samples with inconsistent VDRL and TPHA results, it should be confirmed by another specific treponemal test like FTA-Abs. In view of our study, we conclude that treponemal test like TPHA should be implemented in all the laboratory settings as a confirmatory test for the reactive VDRL samples to accurately diagnose and confirm syphilis infection.

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