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Original Research Article

Analysis of reactivity pattern of venereal disease research laboratory test in a tertiary care hospital

Prabahar P.*, Vijayabhaskar C.

Institute of Venereology, Rajiv Gandhi Government General Hospital and Madras Medical College, Chennai - 600003, Tamil Nadu, India

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***Correspondence:**
Dr. Prabahar P.,
E-mail: drpprabaharmddvl@gmail.com

ABSTRACT

Background: In India, sexually transmitted diseases are one of the major risk factors for acquisition of HIV and infertility. Most of the sexually transmitted diseases are asymptomatic and we rely on cytology, culture, serological evidence and sexual history to diagnose them. VDRL and TPHA are the two most important serological tests in the diagnosis of syphilis. Aim of the study was analysis of quantitative VDRL reactivity pattern and the prevalence of false positive VDRL.
Methods: 7543 patient records were taken. Age, gender, and referral status were recorded. Total records of study population were divided into 2 groups. Group 1- true positive-both VDRL and TPHA Positive. Group II- False positive- VDRL positive and TPHA negative.
Results: Among 7543 cases the ratio of True positive: false positive = 84:16 and in both groups males outnumbered females. In both groups, low titer VDRL took 70%. 61% of false positive cases were in the older age group.
Conclusions: True positives were seen mainly in younger sexually active age group with majority having low titer VDRL (Less than 1:8) emphasizing the need for creating awareness of STDs among this age group and the need for early interventions.

Keywords: Biological false positive VDRL, TPHA, VDRL

INTRODUCTION

In India, sexually transmitted diseases are one of the major risk factors for acquisition of HIV and infertility. Most of the Sexually Transmitted Diseases are asymptomatic and we rely on cytology, culture, serological evidence and sexual history to diagnose them. Syphilis is caused by *Treponema pallidum*, presents with diverse clinical manifestations that occur in distinct stages. As it cannot be readily cultured or stained with simple laboratory method, serology remains the mainstay of laboratory testing for the diagnosis syphilis. The serological test for syphilis includes non-treponemal and treponemal test.[1] VDRL and TPHA are the two most important serological tests in the diagnosis of syphilis. VDRL is the best screening test and quantitative analysis of VDRL is the prognostic indicator.[2]

Aim of the study was to analysis of quantitative VDRL reactivity pattern and the prevalence of false positive VDRL- a retrospective study in a tertiary care hospital.

METHODS

This observational study was conducted in our Institute of venereology, in Rajiv Gandhi Government General Hospital, Chennai, Tamil Nadu, India during the period of January 2016 to June 2016.

7543 cases were tested for syphilis with VDRL and TPHA. Out of 7543 cases, 4599 were males and 2944 were females. The patients were grouped into (1) patients who came for screening without any complaints, (2) patients referred from other specialties and other hospitals (government and private), and (3) Partner screening.

Out of 7543 cases 128 became VDRL reactive (1.7%). All the VDRL reactive cases were tested for TPHA. Among the VDRL reactive cases 110 became positive for TPHA (85.9%) and 18 cases were TPHA negative (14.1%). Out of the 110 cases, VDRL titer was >1: 8 in 37 whereas VDRL was <1: 8 in 73. Only in 3 cases, VDRL titer was high (1: 126). The remaining 18 cases were TPHA negative and diagnosed as biological false positive cases.

Lab Procedure

In above subject groups, Syphilis screening was done by VDRL test. VDRL reactive serum again underwent serial dilution for quantitative analysis. VDRL reactive serum was again tested for TPHA to rule out false positive VDRL. VDRL non-reactive patients were advised to repeat the test after 3 months with sexual abstinence or protected sex.

RESULTS

In this study 7543 patients were screened with VDRL test and 128 (1.7%) turned out to be reactive. VDRL reactivity was higher in male patients (2.3%) as compared with females (0.75%) as shown in Table 1. Patients were grouped as true positive (Group I) who were both VDRL and TPHA positive, and false positive (Group II) who were VDRL positive and TPHA negative.

Table 2 shows quantitative VDRL results based on gender and age group in true positives and false positives. 1.46% (110/7543) of screened population were true positives with 1.96% males and 0.68% females. Among the 18 (0.24%) false positives (group II), 0.35% were males and 0.07% were females. Among the true positive samples 33.6% had VDRL titer >1:8 and 66.4% had titers less than 1:8. True positives were highest in the age group of 20-29 closely followed by >50 years age group. But false positives were observed higher in older age groups with low titer VDRL (<1: 8) in 83% of them.

Table 1: Demographic distribution of new OPD cases in STD.

	New
Male	4599
Female	2944
Total	7543

Table 3 shows marital status of the study groups and Table 4 shows the referral status. Of the total 128 reactive samples, 58.6% were married individuals, of which 49% were true positives and 9.4% false positives. 41.4% of reactive samples were unmarried of which 38.7% were true positives. 36 males gave history of homosexuality. All 36 cases were true positives forming 28% of the reactive samples. Seroprevalence of syphilis considering positive VDRL and TPHA was highest in referral cases from other specialties 58% (75) and followed by direct walk-in patients 34 %(44). In group II as well referrals were higher (72%), followed by direct walk-in (22%). Some of the clinical findings noted in false positive group include balanoposthitis, pearly penile papules, fordyce spots, etc., as shown in Table 5.

Table 2: Comparison of VDRL TITIRE among TRUE positive and FALSE positive VDRL cases with age and sex wise distribution.

	True positive- VDRL+, TPHA+				False positive- VDRL+, TPHA-			
	Male-90		Female-20		Male-16		Female-2	
VDRL	<8	>8	<8	>8	<8	>8	<8	>8
<19	3	1	1	0	1	0	0	0
20-29	8	22	5	0	2	1	0	0
30-39	11	8	4	1	3	0	0	0
40-49	10	4	3	0	0	0	0	0
>50	22	1	9	0	9	0	2	0
Total	54	36	19	1	15	1	2	0

Table 3: Marital status of the study groups.

	Male		Female	
	Married	Single	Married	Single
Group 1	46	44	17	3
Group 2	10	6	2	0

Table 4: Pattern of patient flow to STD OPD.

	Male				Female			
	Direct walk in	Referral	Partner of STD Patient	Total	Direct walk in	Referral	Partner of STD Patient	Total
Group 1	32	57	3	90	12	18	2	20
Group 2	2	14	0	16	0	2	0	2

Table 5: BFP VDRL and clinical correlation.

False positive cases	Male	Female
Old age	9	2
Thalassemia	1	0
Balanoposthitis	1	0
Pearly penile papule	1	0
Fordyce spot	1	0
BPH	1	0
screening	2	0
Total	16	2

DISCUSSION

Syphilis has been referred to as the great imitator due to its wide variety of clinical presentation at one end and asymptomatic latency at the other end.[3] Therefore, prompt diagnosis and treatment is essential not only to lower the transmission rate but also to avoid the late stage complications like paralysis and death due to cardiovascular and neurosyphilis. VDRL is one of the screening tests to make a presumptive diagnosis. The use of only one test is insufficient for the diagnosis of syphilis which has lot of stages with different treatment regimens and, the false positive nontreponemal test results are sometimes associated with various medical conditions. *T. Pallidum* can be demonstrated by dark ground microscope, but the presence of spirochete may be difficult to demonstrate because of inadvertent use of systemic and topical antibiotics and antiseptics. It is not easily cultured and cannot grow in artificial media.[4] Therefore, VDRL and TPHA took a key role in diagnosis, staging and to determine the treatment regime.

VDRL: venereal disease research laboratory test. The basis of VDRL test is the antibody produced by human body when infected with *T. Pallidum*, and the antibody is detected by subjecting the serum to an antigen, which is composed of colourless alcoholic solution of beef cardiolipin, cholesterol and lecithin. As antigen used in nontreponemal test is component of all mammalian cell membranes, damage to the host infection, immunization, pregnancy, aging, or auto immune disorders, antiphospholipid antibody syndrome, drug addiction, liver diseases, malignancies, HIV, burns, recent myocardial infarction, and some febrile illness like malaria, filariasis, tuberculosis can result in false positive results.[5]

VDRL reactive specimens were subjected to quantitative VDRL test with successive twofold dilutions of serum in 0.9% saline and it gives the result in 4-fold increasing order.[6] The VDRL titration is crucial step to staging the syphilis and to determine the treatment. Low titer VDRL are observed in early syphilis, early latent syphilis, late latent syphilis, treated cases, long standing syphilis, burnt out cases, primary syphilis, relapse, reinfection, seroscar, serofast, HIV, baby of syphilitic mother due to maternal transfer of antibody, cardiovascular and neurosyphilis, gumma, and last but not least biological false positive cases.[7] High titre VDRL can occur in secondary syphilis, early latent syphilis, neurosyphilis, gumma, reinfection, rarely some cases of BFP like connective tissue disorder and multiple myeloma.[7]

In the present study VDRL reactivity (including both true positive and false positives) was highest in referrals from other specialties and other primary and secondary care level hospitals as compared to direct walkin. 1.7 % of tested population was found to have reactive VDRL sera, compared to 1% in Safdarjung medical college, Delhi Study.[8] This study showed 1.46% of true positives as compared to only 0.8% in the above Safdarjung study, and 0.24% of false positives which is comparable to 0.2% of false positives in Safdarjung study. In this study, 0.24% of false positives were noted which is like a study from STI unit of Vienna GH, where BFP reactivity was found in 0.24% of all patients.[9] VDRL reactivity was higher in males than females, in contrast to a study from Safdarjung medical college, Delhi, India which showed comparable reactivity in both males and females. Men have greater number of false positive reactions than women in this study which contrasts with Vienna study as well as Jamaican study where a higher female: male ratio was observed in false positives; but is comparable to Safdarjung study.[8].

CONCLUSION

In this study, the incidence of syphilis was 1.46% of total STD OPD attendees of which males outnumbered females. VDRL reactivity was more in the referral cases (68%) from various specialty OPDs emphasizing the importance of screening of referrals. Even though false positive VDRL is very low in total samples but increasing when compared to the true positive cases. The prevalence of false positive VDRL was higher in cases >50 years of age (61%) with 83% having low VDRL titer (less than 1:8). Low titer VDRL required further investigations such as ANA, RF, CXR, Mantoux, etc. to rule out BFP and to find out the staging of syphilis, we should go for cardiology, neurology, dermatology, ortho, and chest physician opinions.[10] The knowledge about the low titer VDRL is very useful for all specialty doctors to refer cases to STD OPD.

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