Various Laboratory Techniques for diagnosing Syphilis- A Comparative Study

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Abstract

Aim: *To compare the effectiveness of POCT and VDRL in the screening of syphilis among high risk group (HRG).*

Methods and Material: *The blood samples were collected from the HRGs' attending the STD OP of IOV from January 2010 to December 2010. The serum specimens from these blood samples were subjected to new point of care test (POCT – Rapid Specific Treponemal) test, Venereal Disease Research Laboratory (VDRL) / Rapid plasma Reagin (RPR) and Treponema pallidum haemagglutination (TPHA).*

Statistical analysis used: *SPSS version 10.0 and EPI 6 package, Chi-square test. To assess the statistical significance at 5% level, a two-sided test was considered.*

Results: *A total of 1131 High risk group (HRG) patients attended. Out of this, 599 (59%) were Men who have Sex with Men (MSM), 402 (35.5%) were Female sex worker (FSW), and 130 (15.5%) were Transgender (TG). The positivity rate of Syphilis was 68 among 599 (11.3%) in MSM, 33 among 400 (8.2%) in FSW and 17 among 130 (13%) in TG by POC test. The overall prevalence of syphilis by POCT test was 10.4% in HRG. The positivity rate of syphilis by VDRL test was 3.3(%) The concordance reactivity of the reactive serum sample tested by POC test was 89% with TPHA and was 33% with VDRL. If VDRL alone were used to screen Syphilis, 2/3 of the positive patients would be missed in this group.*

Conclusions: *The POCT test-screening method is highly sensitive, rapid, cost-effective, and easy to perform. Thus this treponemal test can be used to screen Syphilis to identify the true burden of disease in the community.*

Introduction

The global burden of sexually transmitted infections (STI) has been under-reported because such cases are often asymptomatic, mild or transient. Also, the laboratory screening tests are not reliable at the point of care or primary care level, where most of the people seek medical advice. Genital ulcer disease plays a major role in the transmission of HIV than other STI syndromes.[1] The most common GUD – Syphilis has long-term consequences and congenital transmission causing increased mortality and morbidity.

Clinical diagnosis of Syphilis is complex and often missed because of its latency and atypical clinical presentation except in primary stage which is a short transient period. The laboratory method for screening Syphilis has certain lacunae. The serological methods have been largely unchanged for more than 50 years. The present

VDRL/RPR tests have the sensitivity of less than 50% by unpublished data of CDC.[2,3] These tests VDRL/RPR needs subjective expertise, uses non-specific antigen; besides, reliability of the tests at the point of care or primary care level is questionable and has no uniformity in reporting, due to the lack of standardization of kit and their biological false positivity and false negativity.

With these lacunae, screening Syphilis with VDRL/RPR will not represent the true prevalence of this disease. So the present situation needs a specific test to screen Syphilis at the point of care level which is easy to conduct, less time consuming, objective,, reproducible with high sensitivity and specificity, and cost effective using modern molecular technological development; also it does not have prozone phenomenon. So the POC test for Syphilis was used to screen the Syphilis in HRG, and this test could be compared with VDRL and TPHA tests.

Subjects and Methods

Institute of Venereology (IOV) is a Regional STI Centre designated by NACO for Tamil Nadu, Kerala, Pondicherry, and Lakshadweep. IOV, Tamil Nadu State AIDS Control Society (TANSACS), and AIDS Prevention Control Society (APAC) have conducted a Target Intervention Programme to all the high-risk groups (HRGs). The Ethical Committee of NIE (ICMR), Chennai has given approval for the study.

The Department of Serology of IOV has been participating in VDRL EQAS conducted by WHO regularly for more than 30 years and also participating in related events from NACO's STI-APEX Centre Vardhaman Mahaveer Medical College (VMMC) and Safdarjung Hospital with good performance records.

The cross-sectional study was conducted for a period of one year from January 2010 to December 2010. A total of 1131 HRGs' were screened.

All the HRGs' attending the STD-OP were selected for the study. The clinical and demographic data were recorded. Blood samples were collected using vacutainers and respective sera were separated from all the participants (HRGs). All the HRGs' sera were subjected to POC test, VDRL, and TPHA and were tested as per the manufacturer's instruction. The POC test kit for Syphilis does not show any biological false positive and false negative reaction. The POC (Instachk – Rapid Specific antigen detection kit) test kit was supplied by Transasia Biomedical INTEC Products China. The VDRL test kit was supplied by Institute of Serology, Calcutta, TPHA (Treponema Pallidum Haemagglutination Test) kit was supplied by Omega Diagnostics, Scotland, UK.

The POC test kit (Instachk) for Syphilis was evaluated by NARI – Pune and showed 95% sensitivity and 100% specificity before starting the study. The data analysis was performed by using statistical software SPSS version 10.0 and EPI 6 package. To compare the proportion of cases across the HRGs, Chi-square test was employed. To assess the statistical significance at 5% level, a two-sided test was considered.

Results

A total of 1131 HRGs' attended the STD clinic from January 2010 to December 2010. Among them 599 were MSM, 402 were FSW and 130 were TG. The cases were referred from Non-Governmental Organizations (Fig. 4).

The POC test results, VDRL reactivity status was compared with TPHA and is shown in Table 1. Out of 1131 cases, 118 (10.4%) were positive by POC, 38 (3.3%) were reactive by VDRL, and 105 (9.2%) were positive by TPHA test (Figs. 3 and 5). All VDRL reactive cases were also positive by POC and TPHA (Figs. 1 and 2). The median age of positive cases by POC test was 34 years (range19–65 years) with most of them (32, 28%) were in the age group 26–30 years followed by 26 (22%) between 36 and 40 years. The rate of Syphilis detection is more by POC test (10.4%) followed by TPHA test (9.2%), and then VDRL (3.3%). The proportion of positive cases detected

by VDRL across the HRG among the positive cases detected by POC test is significantly different (P < 0.05; Table 1: P = 0.0165, Chi-square = 8.2). The proportion of positive cases detected by TPHA across the HRG among the positive cases detected by POC test is significantly similar (P > 0.05; Table 1: P = 0.086, Chi-square = 4.88). So the positivity rate between the VDRL and POC test is significantly different as per statistical methods. But the TPHA and POC specific tests are statistically similar.

Table 1 – Comparison of Rapid Specific test, VDRL, TPHA.					
S. no.	HRG	Total no of cases screened for Syphilis	Total no of POC test positive	Total no of TPHA positive	Total no of VDRL positive
1	MSM	599	68 (11.3%)	63 (10.5%)	28 (4.6%)
2	FSW	402	33 (8.20%)	26 (6.4%)	5 (1.2%)
3	TG	130	17 (13%)	16 (12.3%)	5 (3.8%)
Total		1131	118 (10.4%)	105 (9.2%)	38 (3.3%)

The results of quantitative VDRL are given in Table 2. The activity of the disease is more in MSM. Out of 28 reactive samples, 6 were >1:8 dilutions, but in all other HRGs, (FSW and TG) the reactivity was <1:8. Among the 118 cases, 105 (89%) did not complain of any symptoms pertaining to Syphilis. Only 13 had symptomatic GUD and is shown in Table 3. The symptomatic case presentation was more in MSM than in FSW and TG. The incidence of symptomatic patient with positive POC test is 11%, and asymptomatic is 89%. Asymptomatic prevalence was more in TG and FSW (94%) when compared to MSM (85.2%). The proportionate of symptomatic cases detected across the HRG group are similar (P > 0.05). Even though the symptomatic activity seems to be different (14% in MSM, 6% in FSW and 5% in TG), there is no statistical significance (Table 3, P = 0.3282, Chi-square = 2.23).

Fig. 1 – Reactive sample by VDRL test.
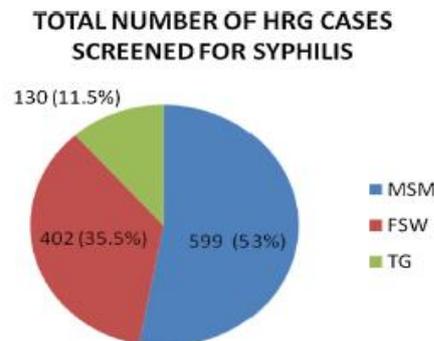

Fig. 2 – Non-reactive sample by VDRL test.

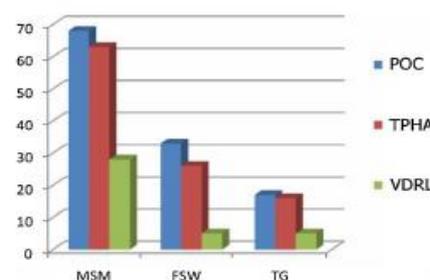Fig. 4 – Total number of HRG cases screened for Syphilis.

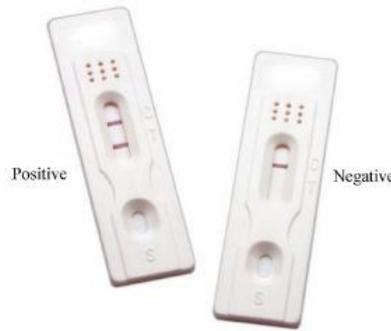Fig. 5 – Comparison of POC, TPHA, and VDRL.

Fig. 3 – POC test (Instachk) – Syphilis.

Table 2 – VDRL Dilution.

S. no.	HRG Group	1:1	1:2	1:4	1:8	1:16	1:32	1:64	Total
1	MSM	14	5	3	1	1	2	2	28
2	FSW	3	2	0	0	0	0	0	5
3	TG	2	3	0	0	0	0	0	5
Total		9	10	3	1	1	2	2	38

Table 3 – Clinical data.

S. no.	HRG	Total no of positives by POC test	Symptomatic	Asymptomatic
1	MSM	68	10 (14%)	58 (85.2%)
2	FSW	33	2 (6%)	31 (93.9%)
3	TG	17	1 (5%)	16 (94%)
Total		118	13 (11%)	105 (89%)

Discussion

This study was undertaken to compare the use of POC and VDRL/RPR in the screening of Syphilis among HRGs in the Serology department attached to Institute of Venereology, Chennai. The prevalence of Syphilis in HRG and their disease activity were also studied. More than 340 million new cases of curable sexually transmitted bacterial mainly Syphilis and protozoan infections occur throughout the world with the largest proportion in the region of south and south-east Asia, followed by sub-Saharan Africa. Most persons with Syphilis tend to be unaware of their infection, and they can transmit the infection to their sexual contacts or in the case of a pregnant woman to her unborn child. Hence, the lab diagnosis plays a key role in the control and prevention of Syphilis. The VDRL/RPR tests are the mainly used tests in the screening of Syphilis and have many disadvantages mainly sensitivity.[4]

The sensitivity of VDRL is 33% when compared to POC in our study. Most of other studies also reported the similar findings 19% by Muic et al.,[5]

by his Bayes' Theorem-Based Assessment of VDRL Syphilis Screening miss rates and less than 50% by Notenboom.[6]

The prevalence of Syphilis in HRG of our study 10.4% is comparable with the data of New York city and Chicago based high prevalence population which is 14.5% and also the concordance of VDRL and POC test is 33% of our study is also comparable to 43.3% between RPR and EIA/CIA of the study done by CDC and Sabibo.[7,8]. STI are more dynamic than other infections prevailing in the community. It is important that such dynamic epidemiological changes in STI are acknowledged and kept track of in a vast and populous developing country like India, particularly in this HIV era[9] with the advent of syndromic case management recommended by NACO even at the primary care level, the incidence of bacterial STI has come down. But asymptomatic stages of Syphilis may be missed if the technical expertise and the sensitivity of the test was low.

Implementation of Syphilis screening programme can be hampered by operational and technical difficulties such as inadequate training, poor supervision, inconsistent quality control, disruption in receiving medical supplies and erratic electricity or refrigeration needed to perform the test or store its reagents by Benzaken et al.[10] Even though the POC test is efficient in diagnosing syphilis, the activity of the disease cannot be detected. So VDRL/RPR test has to be performed followed by positive POC test to stage the disease and to follow-up the case for prognosis. If VDRL is the only test performed to all patients in the study group, yet only 38/1131 (3.3%) would have been diagnosed, but the true prevalence is 118/1131 (10.4%) by POC (Rapid Specific) test. The difference, say 7% of positive cases may be missed. Nearly 80 positive patients for Syphilis may be missed if VDRL is used as a single test to screen Syphilis in our study.

Conflicts of Interest: The author has none to declare.

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