Seroprevalence of syphilis by VDRL test and biological false positive reactions in different patient populations: Is it alarming? Our experience from a tertiary care center in India

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Abstract

Introduction: Many centers for sexually transmitted infections in India perform only a single screening assay for diagnosis of syphilis which may yield biological false positive (BFP) reactions. Aims and Objective: The aim of this study was to determine the true picture of seroprevalence of syphilis and BFP reactions in different patient groups. Materials and Methods: A total of 57,308 serial serum samples obtained over a period of 5 years from different patient groups were screened by venereal disease research laboratory (VDRL) test both qualitatively and quantitatively. VDRL reactive sera were confirmed by Treponema pallidum hemagglutination (TPHA) test. Results: The overall seroprevalence of syphilis by VDRL test was 1.27%, and BFP rate in test population was 0.14%. The rate of BFP reactions among total tested male (0.44%) and female (0.1%) patients differs significantly. Out of 733 VDRL reactive samples, 81 were BFP, i.e., BFP reaction is occurring at a frequency of 11% of the total VDRL reactive samples (ratio of 8:1 for true positives/BFP). Similarly, among antenatal cases, almost 24% of the total VDRL reactive samples were BFP, or for every 116 true positives, there were 37 (almost one-third) BFP. Conclusion: Although the overall seroprevalence of syphilis is low; the frequency of occurrence of BFP reactions is quite alarming. Hence, treponemal test must be used for confirmation of VDRL reactive sera.

Key words: Biological false positive reaction, syphilis seroprevalence, Treponema pallidum hemagglutination, venereal disease research laboratory
yield <1% false positive results making it highly reliable.\(^{[5]}\) However, many centers for sexually transmitted infections (STIs) in India perform only a single screening assay either VDRL or (rapid plasma reagin) RPR for serodiagnosis of syphilis.\(^{[6]}\) Keeping above facts in mind, this study was undertaken to determine the seroprevalence of syphilis by VDRL test and to assess the biological false positivity rate in different patient groups from a tertiary care center in North India.

**MATERIALS AND METHODS**

**Study design**

This study was conducted in the serology laboratory under the department of microbiology of a tertiary care center in North India. A retrospective analysis of results of VDRL and TPHA test was performed on 57,308 serial serum samples, of which 50,030 sera were obtained from female patients and 7278 sera from male patients over a period of 5 years from June 2009 to June 2014. We have further grouped the sera obtained from female patients into antenatal cases (ANCs) \((n = 34492)\) and non-ANC which included STIs clinic \((n = 3072)\) and other outpatient department (OPD)/ward sera \((n = 12,466)\). Sera obtained from male patients were also further categorized into two groups, i.e., those obtained from STI clinic \((n = 2127)\) and from other OPD/ward \((n = 5151)\).

**Syphilis serology**

The clotted blood samples were centrifuged, sera were separated and subjected to heat inactivation at 56°C for 30 min. All the heat-inactivated serum samples were then screened for cardiolipin antibody by the venereal disease research laboratory test (VDRL). The test was carried out using antigen from serologist to the Government of India, Kolkata. VDRL reactive specimens were subjected to quantitative VDRL test with successive 2-fold dilutions of the serum in 0.9% saline. All the sera reactive in qualitative VDRL test irrespective of their VDRL titer were confirmed for antitreponemal antibody by TPHA test by using TPHA Test Kit (Plasmatec Laboratory Products Ltd., Lab21 Healthcare Ltd., 29, Dreadnought Trading Estate Bridport, Dorset). VDRL and TPHA tests were performed according to manufacturer's instructions. All the results of VDRL test were grouped in two categories, i.e., VDRL titer ≥1:8 and <1:8 for evaluation of TPHA results and for assessment of BFP results.

**Statistical analysis**

The statistical analyses were performed using statistical package for social science software (SPSS version 20.0, IBM Corp., Armonk, NY). Fisher's exact test or Chi-square test was used to calculate \(P\) value for analysis of statistical significance of the data. \(P \leq 0.05\) was considered as statistically significant.

**RESULTS**

A total of 57,308 serum samples were obtained from different patient groups, of which 50,030 (87%) sera were obtained from female patients referred from our hospital (34,492 from antenatal OPD, 3072 from sexually transmitted disease (STD) clinic, and remaining from other OPD/ward). A total of 7278 (13%) serum samples were obtained from male patients referred from our hospital (2127 from STD clinic and remaining from other OPD/ward). Female to male ratio was 6.87:1. Overall syphilis seroprevalence by VDRL test was 1.27%, and biological false positivity was found to be 0.14% among the total tested samples. Seroprevalence of syphilis by VDRL test in male patients was found to be 5.02% and BFP rate being 0.44% whereas those in females were 0.74% and 0.1%, respectively \((P < 0.0001)\). The female to male ratio of BFP was 0.2:1 [Table 1].

VDRL reactivity and BFP rates among female patients recruited in the study were found to be 0.44% & 0.1% among ANC patients, 2.08% & 0.61% among STI clinic attendees and 1.21% & 0.06% among remaining female patients respectively [Table 2]. Among male patients recruited in the study, VDRL reactivity was found to be highest 7.1% \((151/2127)\) among STI clinic attendees. Whereas in other male patients included in the study VDRL was positive in 4.15%\((214/5151)\) [Table 3]. Among female patients, out of 368 VDRL reactive sera ≥1:8 titer were found in 76 sera, and 126 sera out of 364 VDRL-positive sera among males had a titer of ≥1:8 \((P = 0.0019)\). All VDRL reactive sera of titer ≥1:8 were found to be positive by TPHA test as well [Table 2].

**DISCUSSION**

Serological tests remain the mainstay of diagnosis of syphilis, nontreponemal tests being screening test.\(^{[3,9]}\) However, it is been shown that the incidence of false positive reactions varies the population being studied.\(^{[10]}\)

| Table 1: Overall VDRL and *Treponema pallidum* hemagglutination positivity in males and females |
|-----------------------------------------------|-----|-----------------|-----|
| Gender                                   | VDRL positive (%) | TPHA positive (%) | BFP (%) |
| Females \((n=50,030)\)                   | 368 (0.74)        | 319 (0.64)        | 49 (0.09) |
| Males \((n=7278)\)                      | 365 (5.02)        | 333 (4.57)        | 32 (0.44) |
| Total \((n=57,308)\)                    | 733 (1.27)        | 652 (1.14)        | 81 (0.14) |

TPHA- *Treponema pallidum* hemagglutination; BFP- Biological false positive
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Similarly, in other study, the seroprevalence in ANC females of 0.44% (153/34492). This result is in congruence with a Delhi-based study, in which the highest seroprevalence of syphilis was found to be 3.5% in overall STD clinic attendees followed by various OPDs, wards (1.43%), and antenatal clinics (0.75%).[11] Similarly, in other study, seroprevalence of syphilis was found to be highest (4.4%, 37/844) in STD clinic attendees, followed by 1% (13/1299) from other OPDs and wards and least in ANC (0.3%, 15/5203).[8] Higher rate in STI clinic attendees could be because of the fact that these patients do exhibit high-risk behavior contributing to the higher seroprevalence as compared to other subgroups of patients. This fact also emphasizes the need for sexual health education for these groups.

In our study, the overall BFP rate was found to be 0.14%. However, in a study conducted by Tankhiwale and Naikwade, the overall BFP rate was 0.47% (61/13,008),[12] which is slightly higher than our study findings. We observed that BFP rate in males was 0.44% and that in females was 0.1%, which is highly significant ($P < 0.0001$). Our result is in congruence with published data from a Delhi-based study where men (0.2%) had a significantly higher number ($P < 0.001$) of BFP reactions than women (0.1%).[11] However, our result is in contrast to Vienna study where BFP was significantly higher in women than in men (0.27% vs. 0.20%, $P < 0.001$).[13] This could be because of the fact that the seroprevalence and BFP rate varies with geographically different areas.

The most notable part here to discuss is that the BFP rate is low if it is calculated from total number of samples tested, however, it needs to be kept in mind that a large number of samples that were tested in our study and the VDRL positivity was

### Table 2: Results of VDRL, *Treponema pallidum* hemagglutination positivity, and biological false positive reactions in different groups of male patients

| Syphilis serology result | STI clinic, n=2127 (%) | Others (OPD/ward), n=5151 (%) | Total males, n=7278 (%) |
|--------------------------|------------------------|-------------------------------|-------------------------|
| VDRL positive            | 149 (7)                | 215 (4.17)                    | 364 (5.02)             |
| VDRL titer               |                        |                               |                         |
| <1:8                     | 91 (4.28)              | 147 (2.85)                    | 238 (3.27)             |
| ≥1:8                     | 58 (2.73)              | 68 (1.32)                     | 126 (1.73)             |
| TPHA positive            | 133 (6.25)             | 200 (3.88)                    | 333 (4.57)             |
| BFP reactions            | 18 (0.85)              | 14 (0.27)                     | 32 (0.44)              |

**TPHA=** *Treponema pallidum* hemagglutination; **BFP=** Biological false positive; **STI=** Sexually transmitted infection; **OPD=** Outpatient department

The overall seroprevalence of syphilis by VDRL test in our study was 1.27% (733/57,308). This compares well with the finding of Bala et al., in which seroprevalence of syphilis was reported to be 1.2%[11] while the overall seroprevalence of syphilis in a study from Maharashtra was found to be 0.7% (90/13,008).[12]

In our study, we found that seroprevalence of syphilis by VDRL test among male patients was 5.02 (365/7278) while that in females was 0.74 (368/50,030), and the difference between the two was highly significant ($P < 0.0001$). It is comparable to the report by Geusau et al.[13]

Moreover, in a study conducted by Tankhiwale and Naikwade, the seroprevalence of syphilis in males and females was 1.73% (44 of 2537) and 0.4% (46 of 10414), respectively.[12] The higher seroprevalence of syphilis in males could be because of the fact that only the suspected patients or high-risk patients or patients attending STI clinic are usually tested for syphilis serology. Whereas, for females, all the ANC attendees are routinely screened for syphilis. Hence, the number of samples from female patients is more, and seroprevalence is less as compared to male patients.

Among various subgroups in our study, the highest seroprevalence among both males and females was found in STI clinic attendees, which was 7.1% (151/2127) and 2.08% (64/3072), respectively, followed by other OPD/ward patients and lowest seroprevalence in ANC females of 0.44% (153/34492). This result is in congruence with a Delhi-based study, in which the highest seroprevalence of syphilis was found to be 3.5% in overall STD clinic attendees followed by various OPDs, wards (1.43%), and antenatal clinics (0.75%).[11] Similarly, in other study, seroprevalence of syphilis was found to be highest (4.4%, 37/844) in STD clinic attendees, followed by 1% (13/1299) from other OPDs and wards and least in ANC (0.3%, 15/5203).[8] Higher rate in STI clinic attendees could be because of the fact that these patients do exhibit high-risk behavior contributing to the higher seroprevalence as compared to other subgroups of patients. This fact also emphasizes the need for sexual health education for these groups.

### Table 3: Results of VDRL, *Treponema pallidum* hemagglutination positivity, and biological false positive reactions in different groups of female patients

| Syphilis serology result | ANC, n=34,492 (%) | STI clinic, n=3072 (%) | Other (OPD/ward), n=12,466 (%) | Total females, n=50,030 (%) |
|--------------------------|---------------------|------------------------|-------------------------------|----------------------------|
| VDRL positive            | 153 (0.44)          | 64 (2.08)              | 151 (1.21)                    | 368 (0.74)                |
| VDRL titer               |                     |                        |                               |                           |
| <1:8                     | 132 (0.38)          | 45 (1.46)              | 117 (0.94)                    | 294 (0.59)                |
| ≥1:8                     | 23 (0.07)           | 19 (0.61)              | 34 (0.27)                     | 76 (0.15)                 |
| TPHA positive            | 116 (0.34)          | 59 (1.92)              | 144 (1.16)                    | 319 (0.64)                |
| BFP reactions            | 37 (0.11)           | 5 (0.16)               | 7 (0.06)                      | 49 (0.1)                  |

**TPHA=** *Treponema pallidum* hemagglutination; **BFP=** Biological false positive; **ANC=** Antenatal case; **STI=** Sexually transmitted infection; **OPD=** Outpatient department
also low. If we correlate BFP with VDRL reactivity, then the impression totally changes. Out of 733 VDRL-reactive samples, 81 were BFP, which means that BFP reaction is occurring at a frequency of 11% of the total VDRL reactive samples, providing us a ratio of 8:1 for true positives: BFP. Similarly, among ANC patients, almost 24% of the total VDRL reactive samples were BFP, or for every 116 true positives, there were 37 (almost one-third) BFP reactions. However, in male patients for every 333 true positive, there were 32 BFP. These figures are in fact quite alarming. It will be more so if we calculate it as a percentage of low titer positives.

Moreover, we found that all the samples with VDRL titer ≥1:8 were true positive. All the BFP samples had VDRL titer <1:8. Thus, we arrive at a conclusion that VDRL titer ≥1:8 can be considered as true positive. However, the point to note here is that only 0.15% (males) and 1.73% (females) of VDRL reactive sera showed titer ≥1:8. Hence, all the remaining cases fall into <1:8 VDRL titer category where TPHA test is must for confirmation of the VDRL test results. Our figures highlight the importance of confirming VDRL reactive sera with treponemal test.

Limitation
We could not include the original illness wise category of the patients coming from STI clinic and other OPD/ward and also the HIV serology status of the patients because of nonavailability of the data.

CONCLUSION
The overall seroprevalence of syphilis is low with the highest seroprevalence being in STD clinic attendees emphasizing the need for sexual health education for these groups. The most notable finding of this study is that the frequency of BFP reactions is quite alarming. Thus, we conclude that the TPHA test must be used for routine confirmation of a positive VDRL test, especially in cases having titer <1:8.

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Conflicts of interest
There are no conflicts of interest.

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