DOI: 10.7860/NJLM/2021/49721:2536

Original Article

Microbiology Section

Biological False Positive Venereal Disease Research Laboratory in Hepatitis B and C Infections in a Tertiary Care Hospital, Delhi, India

SHIWANGI SHARMA[1], MALA CHHABRA[2], NANDINI DUGGAL[3]

ABSTRACT

Introduction: Venereal Disease Research Laboratory (VDRL) test is commonly used for screening of syphilis. In India in some centres, the diagnosis of syphilis is based on VDRL test without confirmation by specific treponemal test resulting in Biological False Positives (BFPs) due to non specific nature of antigen used. Both hepatitis and syphilis are sexually transmitted diseases which are widely prevalent in India. Diagnosing syphilis aptly is important due to its association with various complications and social stigmas.

Aim: To demonstrate the false-positivity of VDRL test in patients with Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infection.

Materials and Methods: This was a cross-sectional observational study in which 420 hepatitis B positive and 208 hepatitis C positive serum samples of patients >18 years of age were tested for syphilis by VDRL and *Treponema pallidum* Haemagglutination (TPHA). VDRL reactive and TPHA negative samples were considered as BFPs. Sera of 90 healthy individuals, which were negative for both Hepatitis B and C were included as control group. The data entry and analysis was done in the Microsoft Excel spreadsheet.

Results: Out of 420 hepatitis B reactive samples, 64 (15.23%) and out of 208 hepatitis C reactive samples, 24 (11.5%) were observed to be BFPs. Of these 64 hepatitis B reactive patients, 46 (71.88%) were females and 18 (28.12%) were males with BFP VDRL test. Similarly, out of the 24 hepatitis C reactive patients, 18 (75%) were females and 6 (25%) were males with BFP VDRL test. BFP was observed to be 1.1% in control group.

Conclusion: The study highlights that HBV and HCV infection are associated with increased chances of obtaining a BFP-VDRL test. If VDRL test is reactive, then reflex testing should be done with a specific treponemal test for a confirmatory diagnosis.

Keywords: Haemagglutination, Reflex testing, *Treponema pallidum*

INTRODUCTION

Syphilis, an ancient sexually transmitted disease, caused by the spirochetes bacterium *Treponema pallidum*, continues to be a public health problem worldwide. According to the Indian Council of Medical Research (ICMR), 5-6% of sexually active adults are suffering from Sexually Transmitted Infections (STIs). In 2015 there were 30,006 cases of Syphilis (16,128 males and 13,878 females) in India [1].

The VDRL is one of the most widely available screening tests for syphilis in India. The World Health Organisation (WHO) recommends the use of a combination of a non treponemal test and a treponemal test for screening and diagnostic purposes [2]. However, still at some centres in India, the diagnosis is made only on the basis of VDRL test without confirming by any specific treponemal test. This may result in false positive (BFP) and false negative results leading to misdiagnosis. This is due to the fact, that the antigen used in non treponemal test is non specific and can result in false-positive non treponemal test results [3].

The VDRL and Rapid Plasma Reagin (RPR) are non treponemal serological tests which essentially do not detect any specific antitreponemal antibodies. The reactivity of these is based on antibodies, both IgM (also known as reagin) and IgG, from the sera of syphilitic donors to non specific cardiolipin-cholesterol lecithin antigens [4]. The antibodies are directed towards cardiolipin, liquid hapten antigen which is part of spirochaetes and is also found in many tissues. These antibody-cardiolipin complex are detected by serological screening tests by using various methods such as complement fixation, precipitation or flocculation techniques [5].

As per the latest estimates by WHO, 40 million people are chronically infected with hepatitis B and 6-12 million people are chronically infected with hepatitis C in India [6]. Thus, the two STIs i.e., syphilis and hepatitis constitute a large portion in the Indian subcontinent. Diagnosing syphilis aptly becomes important because if left untreated, syphilis can cause several complications and may also facilitate the transmission of HIV virus due to the ulcerative nature of this disease [7]. However, misreporting syphilis in patients falsely increases the burden of the disease and is associated with social stigma.

This study was undertaken to detect the VDRL-BFPs in the patients infected with Hepatitis B or C virus. There is limited data available not only from India but also from other parts of the world regarding the same. The aim of this study was to detect the false-positivity of VDRL test in patients with HBV and HCV infection.

MATERIALS AND METHODS

The study was a cross-sectional study conducted from June 2019 to December 2019, in a tertiary care hospital in Northern India. All the samples were received as a part of preoperative workup, by the Department of Microbiology on routine basis (audit), for hepatitis B and C testing from various surgical departments. In this period of seven months, Hepatitis B or C reactive sera was collected (which was adequate in quantity) and was further tested for VDRL and TPHA for the purpose of this study. The confidentiality of the patients was maintained as per Declaration of Helsinki.

Inclusion criteria: Four hundred and twenty (420) hepatitis B positive and 208 hepatitis C positive serum samples of patients

>18 years of age, either sex were included in the study. Sera of 90 healthy randomly selected individuals, who were not related to the cases and were negative for both Hepatitis B and C were included as control group.

Monolisa™ HBsAg Ultra Enzyme Linked Immunosorbent Assay (ELISA) and Monolisa™ HCV Ag-Ab ultra (4th gen ELISA) by BioRad (California, US) were used for detecting hepatitis B and C respectively in the sera of the patients and controls. The hepatitis reactive sera and control sera were further screened for syphilis by VDRL test using antigen obtained from the Indian Institute of Serology, Kolkata, West Bengal, India. VDRL-reactive sera were further subjected to quantitative VDRL test with successive two-fold dilutions up to 1:256 titre. All the sera reactive in qualitative VDRL test were confirmed for antitreponemal antibodies by TPHA test using the IMMUTREP® TPHA kit (Omega diagnostics Scotland, UK) as per the manufacture's kit instructions. Sample which was positive by VDRL and negative by TPHA was considered as BFP. Sample positive by both VDRL and TPHA was considered as true positive.

STATISTICAL ANALYSIS

The data entry and analysis was done in the Microsoft Excel spreadsheet. Means, percentages were calculated and data was analysed accordingly.

RESULTS

Out of 420 hepatitis B reactive sera, the male to female ratio was 2.5:1 (300 males and 120 females) with age distribution between 18-62 years and out of 208 hepatitis C reactive sera the male to female ratio was 4:1 (166 males and 42 females) and with age distribution between 19-65 years. Sera of 90 individuals (45 males and females each) which were negative for both Hepatitis B and C and ages between 19-60 years were included in the study as control.

Of the 420 hepatitis B reactive samples, 10 (4.76%) were reactive by both VDRL and TPHA making them as true positives for syphilis and 64 (15.23%) were reactive by VDRL and negative by TPHA making them as BFPs. Out of these 64 BFPs, 46 (71.88%) were females (mean age 31.25 years) and 18 (28.12%) were males (mean age 34.75 years). In 208 hepatitis C reactive samples, 24 (11.5%) were reactive by VDRL and negative by TPHA, indicating BFPs. Of these 18 (75%) were females (mean age 32.88 years) and 6 (25%) were males (mean age 36.84 years). The demographic distribution of the study group is depicted in [Table/Fig-1].

Age (in years)	Hepatitis B positive BFPs (n=64)		Hepatitis C positive BFPs (n=24)	
	Males (n=18)	Females (n=46)	Males (n=6)	Females (n=18)
18-25	3	10	-	2
26-35	12	26	5	12
36-45	2	6	1	2
46-55	1	3	-	2
56-65	-	1	-	-

[Table/Fig-1]: Demographic profile of the BFP patients.

No true positives were observed in Hepatitis C positive patients in present study. BFP was observed to be 1.1% (1/90, a 45-year-old female) with the titre of 1:2 in control group [Table/Fig-2]. Of the 64 BFPs in hepatitis B reactive samples, VDRL titres in majority of patients were 1:1 (n=30) and 1:2 (n=20). Out of 24 BFPs hepatitis C reactive samples, majority of the patients had titres 1:2 (n=8) and 1:4 (n=6). None of the patients had titres >1:8 in either group. The distribution is depicted in [Table/Fig-2].

VDRL titre	BFPs in Hep B reactive samples (n=64)	BFPs in Hep C reactive samples (n=24)	BFPs in control group (n=1)
Weakly reactive/ doubtful	8	2	-
1:1	30	4	-
1:2	20	8	1
1:4	4	6	-
1:8	2	4	-

[Table/Fig-2]: VDRL titres in hepatitis B and C reactive samples.

DISCUSSION

The male to female ratio in this study for hepatitis B reactive sera was 2.5:1 and for hepatitis C reactive sera was 4:1. These findings were similar to the results obtained by other studies [8,9]. In the control samples, the BFP was obtained to be 1.1%. This finding was slightly higher than the findings of other studies from India, where the BFP in general population was observed to be <1% [10]. However, a recent study published from North India stated BFPs to be as high as 4% in pregnant females [11]. As the antigen used in VDRL test is a component of all mammalian cell membranes, any damage to host tissue by infections, immunisation, pregnancy, age-related changes, or autoimmune diseases can result into BFP reactions in non treponemal tests [3].

In this study, out of 420 hepatitis B reactive samples, 64 (15.23%) were found to be BFP and out of 208 hepatitis C reactive samples, 24 (11.5%) were BFP. Authors did not come across any study comparing the VDRL-BFP in Hepatitis B positive patients. The results of Hepatitis C reactive sera and BFP in VDRL are comparable to a study from Turkey, where the BFP in HCV reactive patients was observed to be 10% [12]. However, the results in present study were higher than the results obtained by other studies. A study from US stated 4% BFP among HCV-infected women [13]. Another study from China showed a BFP rate of 4.2% in HCV reactive patients [14]. In present study, the BFP in hepatitis B positive females was 2.5 times than in males (71.88% vs 28.12%) whereas in hepatitis C positive patients the BFP was thrice in females when compared to the male population (75% vs 25%). These findings were similar to the other studies which have stated that BFPs are more common in females [8,15].

It was observed in present study that the BFP in hepatitis B reactive patients were weak in titres i.e., the majority of the patients had titres ≤1:2 [Table/Fig-1]. Only six of the 64 patients had titres >1:2. In Hepatitis C reactive sera, the BFP in 14 out of 24 patients had titre ≤1:2 and 10 out of 24 patients had titres >1:2 [Table/Fig-2]. None of the patients in either group had the titres >1:8. In absence of clinical, historical, or epidemiologic evidence of syphilis, reactive serologic tests provide only indirect evidence of the disease. Studies have stated that the reactivity in BFP is usually in low dilutions (<1:8), however, in exceptional cases false reactivity in very high titres (up to 1:256) has been reported [16]. Therefore, quantitative titre cannot be used to differentiate between a false positive reaction and true positives. Biological false positivity necessitates a repeat of syphilis testing after 10 weeks, as by that time most BFPs will revert back to VDRL non reactivity [17]. In a study, Nayak S and Acharjya B, have highlighted that at a few STI centres in India, only the VDRL testing is performed and patients having titre ≥1:8 are considered to be suffering from syphilis and are treated for the same. This leads to large percentage of low titre VDRL cases being left untreated due to their non confirmation by a specific treponemal test [18].

The VDRL is a technically demanding test and every step of the procedure is crucial to obtain a reliable result. A substantial amount of experience is required both in performing and interpretation of the results [18]. VDRL has the advantage of being economical

as the antigen is prepared in India making the kit cheaper and readily available. Also, VDRL is an important test to understand the progression of the disease and the response to treatment during follow-ups. However, the diagnosis should always be confirmed with a specific treponemal test after screening with VDRL regardless of its titre or any other non treponemal test, a finding which has been concluded by other studies from India by Bala M et al., and Khan N et al., [10,19].

It is noteworthy here that hepatitis B and C infection have various modes of transmission, whereas syphilis is primarily a STD and hence syphilis is associated with social stigma, especially in our country. Indian studies have stated that STIs are believed to have severe psychosocial consequences both at the individual as well as at the community levels. Those diagnosed with STI live a life with decreased self-esteem as patients repeatedly experience shame, anxiety and embarrassment, constant fear of isolation and even rejection [20].

This study advocates that the diagnosis of syphilis should be done after a confirmatory test. If requisition of only VDRL test is received in the laboratory and if found reactive, the concept of reflex testing should be followed i.e., a confirmatory treponemal test should be done for a definite diagnosis. And lastly, eliciting history of Hepatitis B and C in patients before testing for syphilis may help in understanding the BFPs in VDRL test.

Limitation(s)

The study comprised of a small sample size and passive study was done with regard to the samples obtained in the department. Further prospective studies are required, especially from the Indian subcontinent to generate consolidated data for VDRL-BFPs in Hepatitis B and C positive patients.

CONCLUSION(S)

This study concludes that patients with HBV and HCV infection(s) have increased risk of obtaining a BFP-VDRL test therefore syphilis diagnosis should be confirmed by a specific treponemal test after screening with non treponemal serological tests such as VDRL.

REFERENCES

[1] National Health Profile 2016, Ministry of Health & Family Welfare, Gov't of India.
[2] World Health Organization. Treponemal infections. Technical reports series 674. Geneva: WHO; 1982
[3] Hook EW 3rd, Marra CM. Acquired syphilis in adults. N Engl J Med. 1992;326:1060-69.
[4] Kaur G, Kaur P. Syphilis testing in blood donors: An update. Blood Transfusion. 2015;13(2):197.
[5] Medical Microbiology: A Guide to the Laboratory Diagnosis and Control of Infection. Cruickshank R. Churchill Livingstone; 1973.
[6] National Action Plan Combating Viral Hepatitis in India. 2019.
[7] French P. Syphilis. BMJ (Clinical research ed.), 2007;334(7585):143-47.
[8] Geusau A, Kittler H, Hein U, Dangl-Erlach E, Stingl G, Tschachler E. Biological false-positive tests comprise a high proportion of Venereal Disease Research Laboratory (VDRL) reactions in an analysis of 300,000 sera. Int J STD AIDS. 2005;16(11):722-26.
[9] Patel HK, Patel PH, Nerurkar AB. Seroprevalence of syphilis in patient attending tertiary care hospital, Valsad, India. Int J Curr Microbiol App Sci. 2017;6(6):2702-06.
[10] Bala M, Toor A, Malhotra M, Kakran M, Muralidhar S, Ramesh V. Evaluation of the usefulness of *Treponema pallidum* hemagglutination test in the diagnosis of syphilis in weak reactive Venereal Disease Research Laboratory sera. Indian J STD AIDS. 2012;33(2):102.
[11] Sahota SK, Shakya BK, Arora D, Jindal. Biological false positive rapid plasma reagin tests in pregnant females in North India. Int J Community Med Public Health. 2021;8(3):1217-20.
[12] Sonmez E, Ozerol IH, Senol M, Kizilkaya N, Sahin K, Ozbilge H. False-positive reaction between syphilis and hepatitis C infection. Israel Journal of Medical Sciences. 1997;33(11):724-27.
[13] Augenbraun M, French A, Glesby M, Sanchez-Keeland L, Young M, Greenblatt R, et al. Hepatitis C virus infection and biological false-positive syphilis tests. Sexually Transmitted Infections. 2010;86(2):97-98.
[14] Zhu WF, Lei SY, Li LJ. Hepatitis C virus infection and biological false positive syphilis test: A single-center experience. Hepatobiliary & Pancreatic Diseases International. 2011;10(4):399-402.
[15] Smikle MF, James OB, Prabhakar P. Biological false positive serological tests for syphilis in the Jamaican population. Sexually Transmitted Infections. 1990;66(2):76-78.
[16] Wuepper KD, Tuffanelli DL. False-positive reaction to VDRL test with prozone phenomena. Association with lymphosarcoma. JAMA. 1966;195(10):868-69.
[17] Wiwanitkit V. Biological false reactive VDRL tests: When to re-test? Southeast Asian J Trop Med Public Health. 2002;33(Suppl 3):131-32.
[18] Nayak S, Acharjya B. VDRL test and its interpretation. Indian Journal of Dermatology. 2012;57(1):3.
[19] Khan N, Samal B, Shastri J. Seroprevalence of syphilis and co-infections with Hepatitis B virus, Hepatitis C virus and HIV in a tertiary care hospital in Mumbai. International Journal of Scientific Research. 2018;7(11):39-41.
[20] Passanisi A, Leanza V, Leanza G. The impact of sexually transmitted diseases on quality of life: Application of three validated measures. Giornale Italiano di Ostetricia e Ginecologia. 2013;35(6):722-27.

PARTICULARS OF CONTRIBUTORS:
1. Consultant, Department of IDSP, NCDC, New Delhi, India.
2. Consultant, Department of Microbiology, ABVIMS, Dr. RMLH, Delhi, India.
3. Professor, Department of Microbiology, ABVIMS, Dr. RMLH, Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Mala Chhabra,
Consultant, Department of Microbiology, ABVIMS, Dr. RMLH, Delhi, India.
E-mail: malachhabra@yahoo.co.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 06, 2021
- Manual Googling: Jul 03, 2021
- iThenticate Software: Jul 26, 2021 (10%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Apr 03, 2021**
Date of Peer Review: **May 10, 2021**
Date of Acceptance: **Jul 05, 2021**
Date of Publishing: **Oct 01, 2021**