

# Correlation between Venereal Diseases Research Laboratory titers and CD4 T-lymphocyte count determined by flow cytometry in HIV-infected adults: A 5-year study

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## Abstract

**Introduction:** Venereal Diseases Research Laboratory (VDRL) is one of the key tests for the diagnosis of syphilis; however in HIV-positive individuals, it has been reported to give inappropriate results at times. Thus, this study was conducted to determine if the VDRL test titers vary with the severity of immunosuppression as determined by CD4 cell count. **Materials and Methods:** A total of 2630 samples from HIV-positive adults were tested by qualitative and quantitative VDRL test and if reactive, by *Treponema pallidum* hemagglutination (TPHA) test. CD4 cell counts were determined at the same time by flow cytometry (BD FACSCount™ system). Correlation between CD4 T-lymphocyte cell count and VDRL titers was sought for. **Results:** Nearly 6.7% (176/2631) of individuals were VDRL reactive, males more than females (7.6% vs. 5.1%,  $P = 0.014$ ). Four of the VDRL-reactive patients were found negative by TPHA test and were excluded from further study. VDRL titers ranged from weakly reactive to being reactive at 1:128 (median = 1:2). The CD4 cell count ranged from 23 cells/ $\mu$ l to 883 cells/ $\mu$ l (median = 276 cells/ $\mu$ l, mean =  $323.9 \pm 200.9$ ). Pearson's coefficient of correlation ( $R$ ) between CD4 cell count and VDRL titers was calculated to be 0.0559; coefficient of determination ( $R^2$ ) was 0.0031. **Conclusions:** Although the correlation coefficient shows a positive correlation, the association was very weak. Therefore, CD4 cell count cannot be expected to influence VDRL titers in HIV-positive adults significantly.

**Key words:** CD4 cell, HIV, syphilis, Venereal Diseases Research Laboratory

## INTRODUCTION

HIV infection in India and several other regions of the world has reached epidemic proportions, yet too little is known regarding the disease and its interaction with other infections.<sup>[1]</sup> One relationship that has been strongly suggested is that the existence of sexually transmitted infections (STIs) is positively associated with the risk of acquiring HIV infection, probably due to high-risk sexual behavior and genital ulceration.<sup>[2]</sup> It has also been

suggested that syphilis may cause transient increase in viral load and decrease in CD4 cell count that resolve once the infection is treated.<sup>[3]</sup> Likewise, HIV infection worsens the outcomes of several other infections and makes an individual more susceptible to a large majority of infections by inducing an immunocompromised state, leading

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to deficiency of both cell-mediated and humoral immunity.

Syphilis is one STI which is frequently associated with HIV, and the relation between the two is often described as complex. HIV, in the patients of syphilis, may influence the presentation, diagnosis, disease progression as well as the treatment of syphilis.<sup>[4]</sup> Laboratory diagnosis for syphilis involves direct demonstration of *Treponema pallidum*/antigens/nucleic acids or serological detection using nontreponemal and treponemal tests. Nontreponemal and treponemal serologic tests are most frequently used. The interaction between syphilis and HIV makes interpretation of these tests uncertain. Venereal Diseases Research Laboratory (VDRL) test may show unusual serological responses in HIV-infected patients with syphilis.<sup>[5]</sup> Immunoglobulin levels may be high in HIV-positive individuals due to non-specific polyclonal B-cell activation, yet the levels of specific immunoglobulin may remain deficient; this may potentially influence the results and titers of serological testing of infectious diseases in HIV-positive individuals.

Therefore, this study was aimed to determine the seroprevalence of syphilis in HIV-positive adults as well as to find whether the severity of immunosuppression (as determined by CD4 cell count) influences the VDRL titers.

## METHODS

The study was a retrospectively designed observational and analytical study spanning a period of 5 years (2010–2014). The study involved adults (18 years or above) while maintaining their confidentiality. It was conducted at a premier tertiary care hospital and medical college in the capital city of India, New Delhi. This hospital has an antiretroviral treatment (ART) center along with HIV testing laboratory with an Integrated Counseling and Testing Center (ICTC) and State Reference Laboratory.

For HIV testing, the patients presenting to the ICTC were provided pretest counseling and informed consent was taken. They were ensured of the confidentiality of their test results. Three milliliters of blood sample was collected in plain Vacutainer® vial (Becton, Dickinson and Company, USA) and allowed to clot. Serum was separated by centrifuging at 2000–2500 rpm for 15 min. The HIV testing was done strictly following the Strategy III of National AIDS control Organization, India. Briefly, each specimen was initially tested by one rapid

immuno-chromatographic test (RIT); if it tested positive, the specimen was further tested by two more tests (RIT and/or ELISA). Reactive result was given only if all the three tests were reactive. Patients testing reactive for HIV antibodies at the ICTC were given post-test counseling and referred to the ART center.

A total of 2630 samples from HIV-positive adults were tested for syphilis in the Serology laboratory during this period. Three milliliters of blood sample was received in plain Vacutainer® vials, clotted, and sera separated. All samples were tested by VDRL test initially. Briefly, the sera were inactivated by heating at 56°C for 30 min in a water bath. A volume of 0.05 ml of inactivated sera was taken into one well each; 1/60<sup>th</sup> ml of the freshly prepared cardiolipin antigen (Serologist to Government of India, Kolkata, India) was then added to each well and rotated at 180 rpm for 4 min. The slide was then viewed under low-power objective of a microscope for flocculation. Reactive samples were then subjected to quantitative test by preparing successive 2-fold dilutions of the serum in 0.9% saline and repeating the procedure. The highest dilution showing flocculation was considered as reactive titer. Following manufacturer's instructions, *Treponema pallidum* hemagglutination (TPHA) test (Plasmatec Laboratory Products Ltd.) was done for samples that were reactive or weakly reactive by VDRL test. This was done to rule out the possibility of biological false-positive reactions (BFP).

Three milliliters of whole blood was collected for CD4 cell counts in EDTA Vacutainer® vials (Becton, Dickinson and Company, USA), and CD4 cell counts were determined by flow cytometry (BD FACSCount™ system; Becton, Dickinson and Company, USA) strictly following the manufacturer's instructions. Data regarding CD4 cell counts were retrieved for patients testing reactive or weakly reactive by VDRL test and also positive by TPHA test.

## Statistical analysis

The CD4 cell counts and VDRL titers were analyzed and presented as percentages of proportions, median, mean, range, interquartile range (IQR), and standard deviation. Statistical significance of difference in proportions was calculated using Chi-square test;  $P < 0.05$  was considered statistically significant. Pearson's coefficient of correlation ( $R$ ) was calculated to determine the degree of correlation between CD4 cell counts and VDRL titers; coefficient of determination ( $R^2$ ) was also calculated.

## RESULTS

Over a period of 5 years, 2630 HIV-positive adults were included in the study. These comprised 1674 (63.7%) male, 927 (35.2%) female, and 29 (1.1%) transgender patients. Male:female:transgender ratio was 1:0.553:0.017. Year-wise distribution of patients is presented in Table 1. The age of the patients ranged from 18 years to 82 years (median = 32 years, IQR = 27–36 years). Age-wise distribution of CD4 cell count and VDRL titers is detailed in Table 2.

Of these patients, 6.7% (176/2630) tested reactive by the VDRL test. VDRL reactivity was significantly higher among male patients as compared to females (7.6% vs. 5.1%,  $P = 0.014$ ). Majority of the patients (154/176, 87.5%) had titer  $\leq 1:8$ ; while only four patients had titers of 1:128. All the VDRL-reactive samples were retested by TPHA test; barring four samples, all VDRL-reactive samples were also reactive by TPHA test. Three of these TPHA-negative samples were weakly reactive, while one was reactive at 1:2 dilution; these were excluded while determining correlation between CD4 cell count and VDRL titers.

CD4 cell count is done for all the newly identified HIV-positive patients and repeated at regular intervals. Therefore, it was ensured that only the CD4 cell values calculated at the time of VDRL/TPHA testing were considered. The CD4 cell count ranged from 23 to 883 cells/ $\mu\text{l}$  (median = 276 cells/ $\mu\text{l}$ ; mean  $323.9 \pm 200.9$  cells/ $\mu\text{l}$ ). Based on the CD4 cell levels, patients were divided into five groups [Table 3]. A large majority of patients had CD4 cell counts from 200 to 350 cells/ $\mu\text{l}$ , followed by patients with cell counts between 51 and 200 cells/ $\mu\text{l}$ . The medians as well the ranges of VDRL titers were similar for all the groups [Table 3].

Pearson coefficient of correlation ( $R$ ) between CD4 cell count and VDRL titers was calculated to be 0.0559 ( $P = 0.466$ ). The value of  $R^2$ , the coefficient of determination, was 0.0031. Figure 1 shows the graphical linear relation between these two variables.

## DISCUSSION

The study spanned a period of 5 years, involving a large number of HIV-positive adults. There were greater number of male HIV-positive patients as is usually described.<sup>[1]</sup> Around 7% of these individuals tested reactive by VDRL test, and the males showed significantly higher reactivity as compared to the females. This has also been reported

**Table 1: Year-wise distribution of HIV-positive patients included in the study and their Venereal Diseases Research Laboratory status**

Year	Males		Females		Transgenders		Total	
	n	VDRL reactive	n	VDRL reactive	n	VDRL reactive	n	VDRL reactive
2010	208	19 (9.1)	124	6 (4.8)	2	0	334	25 (7.5)
2011	274	32 (11.7)	115	14 (12.2)	12	1 (8.3)	401	47 (11.2)
2012	414	28 (6.8)	221	10 (4.5)	4	0	639	38 (5.9)
2013	377	30 (8.0)	212	11 (5.2)	4	0	593	41 (9.9)
2014	401	19 (4.7)	255	6 (2.4)	7	0	663	25 (3.8)
Total	1675	128 (7.6)	927	47 (5.1)	29	1 (3.4)	2630	176 (6.7)

Figures in parentheses indicate percentages.  
VDRL=Venereal Diseases Research Laboratory

**Table 2: Age-wise distribution of CD4 cell count/ $\mu\text{l}$  and Venereal Diseases Research Laboratory titers**

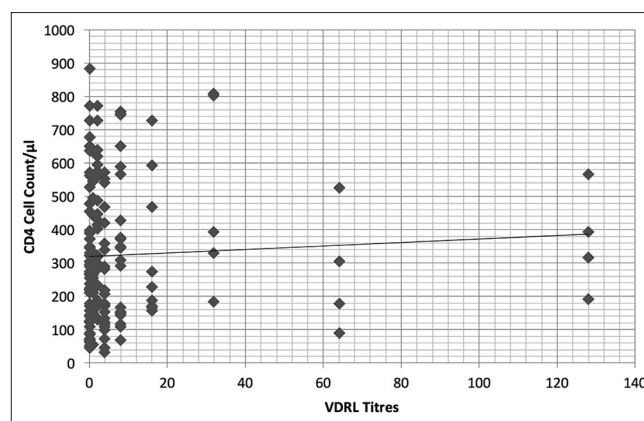
Age group	n	Mean CD4 cell count/ $\mu\text{L}$ ( $\pm$ SD)	Median VDRL titers (IQR)
$\leq 30$	77	365.3 $\pm$ 209.2	2 (WR-64)
31-40	60	349.8 $\pm$ 193.4	2 (WR-128)
41-50	19	322.2 $\pm$ 178.8	2 (WR-128)
51-60	12	310.8 $\pm$ 215.3	1 (WR-8)
$\geq 61$	4	247.3 $\pm$ 118.1	1 (WR-8)
Total	172	323.9 $\pm$ 200.9	2 (WR-128)

WR=Weakly reactive; IQR=Interquartile range; SD=Standard deviation;  
VDRL=Venereal Diseases Research Laboratory

**Table 3: Relationship between CD4 cell count and median Venereal Diseases Research Laboratory titers**

CD4 count	Number of patients	Median CD4 cell count/ $\mu\text{l}$ (IQR)	Median VDRL titers (range)
<50	6	39 (33-45)	2 (WR-4)
51-200	51	133 (89-170)	2 (WR-64)
200-350	57	276 (234-316)	1 (WR-128)
350-500	23	419 (390-453)	2 (WR-128)
>500	35	635 (567-729)	2 (WR-128)

IQR=Interquartile range; WR=Weakly reactive;  
VDRL=Venereal Diseases Research Laboratory



**Figure 1: Linear relation between CD4 cell count and Venereal Diseases Research Laboratory titers. Note: Titer "0" suggests weakly reactive**

previously.<sup>[6]</sup> Transgenders had the lowest prevalence of syphilis. Though there was statistically significant

difference between the year-wise distribution of seroprevalence of syphilis ( $P < 0.0001$ ), no specific trend emerged over a period of 5 years. The overall seroprevalence of syphilis in HIV-positive individuals was highest during 2011 (11.2%) and lowest during 2014 (3.8%) [Table 1]. In contrast, a rising trend in syphilis seroprevalence among HIV-positive individuals has been reported by Sethi *et al.* from Chandigarh, India, from 2006 to 2011, possibly due to increase in the cases of secondary syphilis.<sup>[7]</sup>

The rate of BFP among HIV-positive adults was low, only 4 out of 176 (2.3%) VDRL-reactive patients showing negative reaction in TPHA test. However, as noted by Rompalo *et al.*, false-positive nontreponemal antibody tests may be encountered more frequently in the HIV-positive individuals and may be seen in up to 11% of cases.<sup>[8]</sup>

It has been shown previously that in HIV-positive individuals, the VDRL test may not always give appropriate results. Hicks *et al.*<sup>[9]</sup> and Augenbraun *et al.*<sup>[10]</sup> have reported an increased rate of negative serological tests in both primary and secondary syphilis. Patients may even present with the typical features of primary or even secondary syphilis, but still have been reported to have negative nontreponemal and treponemal antibody results.<sup>[9]</sup> Increased false-negative nontreponemal antibody tests due to the prozone effect have been reported by Haslett and Laverty<sup>[11]</sup> and Jurado *et al.*<sup>[12]</sup> Despite these unusual serologic responses in HIV-infected patients, the Centers for Disease Control and Prevention recommends that the diagnosis and interpretation of the results of both treponemal and nontreponemal serologic tests for syphilis should be the similar in HIV-infected patients as in the general population.<sup>[13]</sup> However, this research was not aimed at evaluating VDRL as a diagnostic modality for syphilis in HIV-positive individuals. As mentioned earlier, several researchers have already attempted that.

Akinpelu *et al.* have previously found that there are no significant differences in the serum immunoglobulin (IgG and IgM) levels when HIV individuals with low CD4 cell counts (<200 cells/ $\mu$ l) were compared with individuals with higher CD4 cell counts (>200 cells/ $\mu$ l).<sup>[14]</sup> In contrast, Lugada *et al.* found levels of IgA, IgG, and IgG1 to vary between HIV-negative and HIV-positive individuals.<sup>[15]</sup> Weakening immune response may even lead to false-negative results in HIV diagnostic tests detecting anti-HIV antibodies;<sup>[16]</sup> thus, it may also influence VDRL test results. Thus, we aimed to determine the effect of these possible

immunological variations on the VDRL titers by correlating them with the marker of severity of immunosuppression, the CD4 cell count. Despite thorough search through various indexing sites and individual journal sites, similarly designed studies were not found.

We found the Pearson's correlation coefficient ( $R$ ) to be 0.0559. Although the correlation coefficient shows a positive correlation (indicating VDRL titers are slightly higher in patients with higher CD4 cell count), the association is very weak. The coefficient of determination ( $R^2$ ) was 0.0031, which signifies that the total variation in VDRL titers can be explained poorly by the linear relationship between CD4 cell count and VDRL titers.

It may be worth mentioning that regardless of the CD4 cell count levels, the median and the range of VDRL titers were almost similar [Table 3]. The range was wide, mostly from weakly reactive to reactive at 1:128 dilutions; further negating the idea that CD4 cell count and thus the severity of immunodeficiency may influence the VDRL titers.

## CONCLUSIONS

However, our study had one limitation; classification was not done according to the stages of syphilis, which may influence the VDRL titers. Nevertheless, due to inadvertent randomization and grouping of all patients together, our study provides sufficient reasons to believe that VDRL titers do not correlate significantly with CD4 cell count in HIV-positive adults.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. National AIDS Control Organization. State Fact Sheets March, 2014; 2014. Available from: <http://www.naco.gov.in>. [Last accessed on 2016 Jun 16].
2. Royce RA, Seña A, Cates W Jr., Cohen MS. Sexual transmission of HIV. *N Engl J Med* 1997;336:1072-8.
3. Zetola NM, Klausner JD. Syphilis and HIV infection: An update. *Clin Infect Dis* 2007;44:1222-8.
4. Golden MR, Marra CM, Holmes KK. Update on syphilis: Resurgence of an old problem. *JAMA* 2003;290:1510-4.
5. Lynn WA, Lightman S. Syphilis and HIV: A dangerous combination. *Lancet Infect Dis* 2004;4:456-66.
6. Sharma S, Chaudhary J, Hans C. VDRL v/s TPHA for diagnosis of syphilis among HIV sero-reactive patients in a tertiary care hospital. *Int J Curr Microbiol Appl Sci* 2014;3:726-30.

7. Sethi S, Mewara A, Hallur V, Prasad A, Sharma K, Raj A, *et al.* Rising trends of syphilis in a tertiary care center in North India. *Indian J Sex Transm Dis* 2015;36:140-3.
8. Rompalo AM, Cannon RO, Quinn TC, Hook EW 3<sup>rd</sup>. Association of biologic false-positive reactions for syphilis with human immunodeficiency virus infection. *J Infect Dis* 1992;165:1124-6.
9. Hicks CB, Benson PM, Lupton GP, Tramont EC. Seronegative secondary syphilis in a patient infected with the human immunodeficiency virus (HIV) with Kaposi sarcoma. A diagnostic dilemma. *Ann Intern Med* 1987;107:492-5.
10. Augenbraun M, Rolfs R, Johnson R, Joesoef R, Pope V. Treponemal specific tests for the serodiagnosis of syphilis. Syphilis and HIV study group. *Sex Transm Dis* 1998;25:549-52.
11. Haslett P, Lavery M. The prozone phenomenon in syphilis associated with HIV infection. *Arch Intern Med* 1994;154:1643-4.
12. Jurado RL, Campbell J, Martin PD. Prozone phenomenon in secondary syphilis. Has its time arrived? *Arch Intern Med* 1993;153:2496-8.
13. Workowski KA, Berman S, Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;59:1-10.
14. Akinpelu OO, Aken'ova YA, Arinola OG. Levels of immunoglobulin classes are not associated with severity of HIV infection in Nigerian patients. *World J AIDS* 2012;2:232-6.
15. Lugada ES, Mermin J, Asjo B, Kaharuza F, Downing R, Langeland N, *et al.* Immunoglobulin levels amongst persons with and without human immunodeficiency virus type 1 infection in Uganda and Norway. *Scand J Immunol* 2004;59:203-8.
16. Li Y, Zhao JK, Wang M, Han ZG, Cai WP, Zheng BJ, *et al.* Current antibody-based immunoassay algorithm failed to confirm three late-stage AIDS cases in China: Case report. *Virology* 2010;7:58.

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**30 PEARLS OF MALE GENITAL DERMATOLOGY**

Dr Anthony hall, associate professor, school of medicine, Deakin University, Australia

21. Genital dysesthesia is distressing, under reported and challenging to manage. Aim to reduce severity of symptoms not necessarily cure.
22. Penile intra epithelial neoplasia is the most important pre malignant genital skin disease. It presents as red macule, papule or plaques which has a low threshold for genital skin biopsy.
23. Bowenoid papulosis appears as skin colour pink or red smooth papules rather than pigmented verrucous papules.
24. Penile cancer is partly preventable disease and is a dermatology issue.
25. Penile cancer is rarely seen if a person is circumcised at birth. It may occur after circumcision for lichen sclerosis.
26. Risk of penile cancer is reduced by treating inflammatory and ulcerative genital disease, limiting genital HPV infection, treating phimosis, preventing ultraviolet exposure and not smoking.
27. Treatment of extra mammary Paget's disease of genitalia requires multi disciplinary approach. Treatment involves more than wide local excision.
28. Genital melanotic macules (genital melanosis) needs to be differentiated from melanoma.
29. Exclude Crohn's disease in chronic genital edema (lymphedema).
30. Long term follow up is essential when a definitive diagnosis is not possible.